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Status Report on Experiment AD-4/ACE

Biological Effectiveness of Antiproton Annihilation

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Summary

Our experimental proposal to study the biological effect of antiprotons was approved by the SPSC in January of 2003 for beam time in the run cycle of 2003. So far during the summer of 2003 AD-4 has received 10 shifts of beam in three independent blocks of time. These shifts were used to perform an initial experiment to establish the correct dose range for meaningful biological exposures, to develop and enhance our dosimetry capabilities, and to perform the first full biological sample irradiation. This document describes these experiments in detail and highlights the problems, challenges, and achievements of our collaboration during this time.

We also comment on the upcoming final run time for this year and present an outlook for the future, detailing a program for a possible continuation in 2004.

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Introduction

The use of ions to deliver radiation to a body for therapeutic purposes is advantageous because the profile of deposited energy peaks at the end of range of the charged particle rather than near the surface as is the case with photon based therapy. This is particularly important for deep-seated tumors or tumors located near radiation sensitive regions that must be spared. Furthermore, the biological effectiveness of charged particle radiation varies widely with the density of ionization or LET (linear energy transfer) of the particle as it moves through the body, which depends on the charge and velocity of the ion. These facts have supported the development of proton and heavy ion therapy centers. Alternatively, antiprotons can also be used to deliver radiation to the body in a controlled way and may have additional advantages over other types of radiation currently used in radiation therapy. The slowing down of antiprotons is similar to that of protons except at the very end of range beyond the Bragg peak. When the antiprotons stop they annihilate producing a variety of low and high-energy particles. The relatively low energy particles deposit biologically effective high LET radiation in the immediate vicinity of the annihilation point. The high-energy pions, muons, and gammas leave the body and have the potential to be used for imaging.

Gray and Kalogeropoulos [1] estimated the additional energy deposited by heavy nuclear fragments within a few millimeters of the annihilation vertex to be approximately 30 MeV. While this is small compared to the total annihilation energy of 1.88 GeV, for biological purposes it can be very significant, especially considering that the energy is delivered in the form of high LET radiation resulting from heavy fragments and recoils depositing all their energy in a localized region around the annihilation vertex.

In 1985, Sullivan [2] measured the relative magnitude of the enhanced energy deposition at the Low Energy Antiproton Ring (LEAR) at CERN, but did not measure the biological effect. Our experiment AD-4/ACE [3,4] is the first to aim at a direct measurement of the biological effects of antiproton annihilation. At this time the experiment can only be done at CERN where the AD (Antiproton Decelerator) has a low energy, mono-energetic beam of antiprotons able to deliver a biologically meaningful dose at an appropriate dose rate.

The main challenge in the design of this experiment is obtaining the maximum of biological information with the limited number of antiprotons available. The experimental design aims to capture enough data for an initial evaluation of the potential for radiotherapy using antiprotons. It is clearly not all that is needed for a definitive assessment of possible therapeutic applications of antiprotons, but it will determine if further studies are warranted.

The experiment uses a beam of 300 MeV/c (46.8 MeV) antiprotons from the AD extracted into a biological sample of live cells. The biological sample is contained within a tube that is designed to hold dispersions of the live cells in a semi-solid biological culture medium. This tube is placed within a phantom situated in air at the end of the DEM beam line. The phantom consists of a refrigerated glycerin and water solution of the same density as the gelatin and sample tubes containing the cells and is used to maintain the cells at 2 degrees C. This ensures that at any given depth, the stopping power was independent of lateral position and thus avoids any artifacts that could result from scattering of antiprotons from points outside the gel.

The quantitative cell survival studies involve counting the number of colonies that grow during an incubation period after irradiation. Prior to obtaining approval for the experiment we performed a demonstration/test experiment at TRIUMF using a 70

MeV proton beam. The analysis method is described in detail in reference 5. The analysis of cell survival at serial 1 mm depths along the beam central axis enables us to determine the lethality of antiprotons as a function of depth along the path of antiprotons.

Comparing biological effectiveness of antiproton annihilation in the peak versus plateau regions of the stopping ionization distribution will give us a measurement of potential differentials in "biological" dose in the tumor and surrounding normal tissues for a therapeutic beam of antiprotons. In other words, the questions we are addressing with our experiment are the following: *"If we compare two particle beams, i.e. protons and antiprotons, having the same physical characteristics (energy, momentum distribution, beam geometry) and delivering identical dose to the entrance channel, by how much will the biological effectiveness of the antiproton stopping peak be enhanced by the densely ionizing annihilation products? Will this enhancement be significant enough to make antiproton beams potentially useful for tumor treatment?"*

Cell survival is a direct measurement of the net effect of all the different ionization species along the antiproton path. The response relative to both protons and ^{60}Co gamma radiation is used to standardize the biological effectiveness of antiprotons. The possible peripheral biological effects of the non-localized mixed radiation fields away from the point of annihilation can be measured in cell samples located at appropriate distances from the region of annihilation, either radial or distal (beyond the Bragg Peak).

Description of experimental work performed June – October 2003

General remarks: To obtain the maximum of information with a minimum of available antiprotons we concentrated in this first experiment on a very simple set-up in which living cells suspended in a gel growth medium are exposed to an antiproton beam of a specific fluence. In the axial direction this beam produces a dose profile showing a distinct peak at the end of range (the Bragg peak) similar to protons. The goal of the experiment is to determine the biological response to this dose distribution by measuring cell survival fractions along the beam axis. The following issues are highly relevant to this type of experiment:

- The method of comparing Biological Effective Dose Ratios (BEDR) of antiprotons to protons rather than measuring an absolute value of the Relative Biological Effectiveness (RBE) makes us largely independent of any precise knowledge of absolute dose (see reference 4 and 5 for details). Even though, since the biological response of cells is highly non-linear, a complete knowledge of particle fluence entering the cell samples is absolutely important for achieving high quality data. Too high particle fluence can easily result in an overkill situation (as was the case in our initial test experiment described below) and thereby yield data, which cannot be used to extract biologically meaningful data. Additionally, the highly non-linear response mandates that the dose delivered to a sample slice is as constant as possible throughout the slice in both radial and axial directions. Because survival is a (negative) exponential function of dose, a non-uniform dose throughout the sample (slice), whether the variation is axial or radial, would result in a disproportionate number of surviving cells coming

from the region in the sample where the dose is lowest. This problem becomes more severe at higher doses, and it is the higher doses that are most influential in determining biological effectiveness. In fact, at high enough doses, virtually all the measured survivors might come from the 5 or 10% of the sample volume that receives the lowest dose. And if that dose is, say, 20% lower than the average dose to the slice, the measured biological effect will be representative not of the average dose but of a dose which is 20% below that. The consequence is a serious underestimation of the actual biological effectiveness of the radiation tested.

- To minimize the effect of non-linear response to dose in the radial direction we typically aim for a beam spot size sufficiently large to assure radial variations of the beam intensity across the area of the target to be less than 5%. For our sample tube diameter of 6 mm this requires a sigma of a Gaussian beam spot in both x and y of 0.94 cm as ideal running conditions, which can be achieved if the AD operates at nominal emittance, i.e. optimum electron cooling performance.
- As the fluence of particles reaching the sample tube scales as the inverse square of the beam spot size, this is a critical parameter and needs to be known before the irradiation with sufficient accuracy to predict a correct range of doses for the experiment.
- In axial direction the situation is even more complex. For protons the dose versus depth can be measured, but for antiprotons this profile is not known experimentally. Therefore we use accurate fluence measurements together with Monte Carlo simulations to predict the physical dose vs. depth. It appears from our observations that this may lead to reasonably accurate predictions for required irradiation times, but we have additionally initiated an experimental study of TLD response to mixed radiation and high LET fragments from annihilation. We will describe this in more detail below.
- Last but not least, the method of taking 1 mm thick samples from our cell tubes requires the axial width of the Bragg peak to be sufficiently large. As a pristine Bragg peak (for a mono-energetic beam at 50 MeV) is only 1.5 mm wide at the FWHM value, we added a three-step degrader to the system to achieve a spread-out Bragg peak (SOPB) of 2.8 mm width.

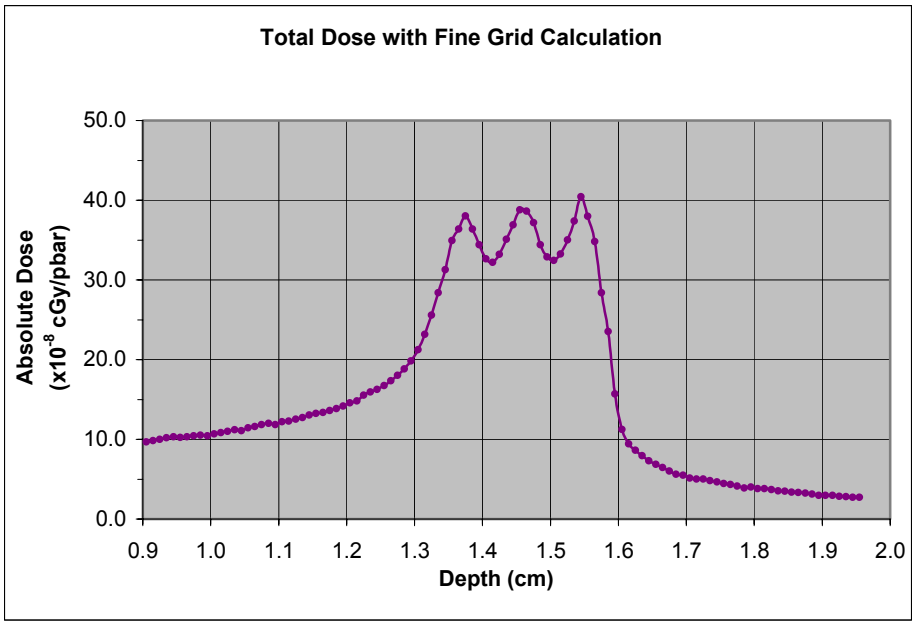

Fig. 1: Monte Carlo calculation of the axial energy deposition of antiprotons entering the dosimetry phantom after passing through our stationary three-step degrader

For the time structure of the beam delivered from the AD the standard method of rotating a modulator plate with varying thickness in the beam is not applicable. Using degraders of different thickness for a certain percentage of the shots in a given experiment is difficult considering that some experiments only need very few shots. Analyzing the lower dose irradiations performed in the experiments to date showed that a 10 – 15% uncertainty in dose across the Bragg peak would result from such a design.

We therefore designed a stationary degrader system consisting of a fine geometric structure (< 1mm), which had three thicknesses (1, 1.8, 2.6 mm) with relative weightings in area of 41, 31, and 28 % respectively. According to Monte Carlo studies this would result in a dose depth profile as shown in figure 1. Immediately behind the degrader we could observe a shadow of the degrader geometry in the radial direction, reflecting an intensity variation in the beam according to position, which then could translate to a dose variation in radial direction. The angular straggling of the particles passing through the degrader will wash out this effect after a short distance in air (and even more so after the beam enters the material of the phantom and sample tube).

This has been confirmed by irradiating GAF chromic film placed both perpendicular to the beam and axially in the beam at the actual distance after the degrader used in the experiment (19 cm). Initial inspection of the film using a standard scanner indicates no significant axial or radial structure due to the degrader in the dose profile (figure 2a and b). These results are supported by preliminary model calculations of the set-up using a simplified 1-dimensional two-step degrader. As shown in figure 2c the effect of the degrader is visible directly after exiting the degrader but is completely washed out by the radial straggling within a few centimeter of flight in air.

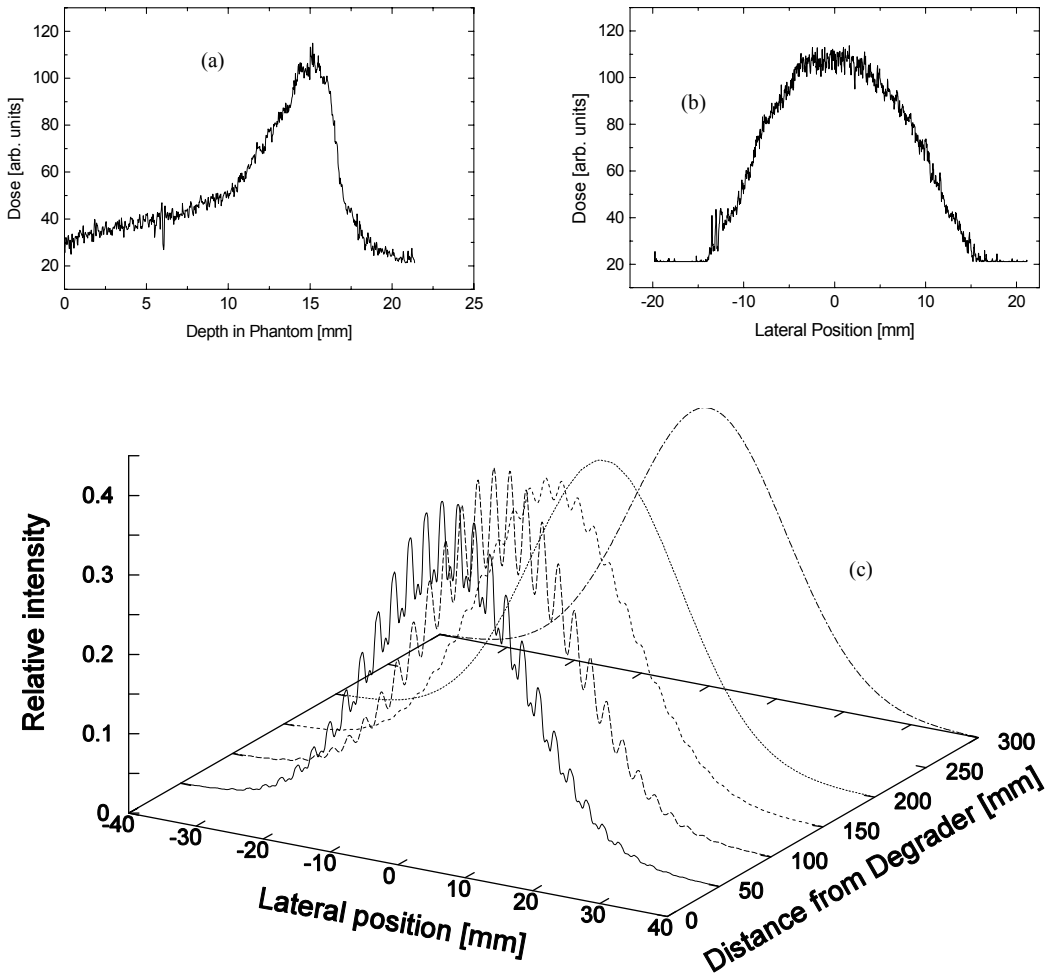

Fig. 2: (a) Axial and (b) radial beam profile as measured with GAF chromic film and (c) results from Monte Carlo Simulation of the set-up (see text).

Run period A – June 8 – 9, 2003: Considering the issues described above, we developed a plan for the first run period consisting of two periods of two shifts each, following each other back to back. The first day was dedicated to beam development and dosimetry studies. During this period we spent most of the time attempting to get a proper beam tune in the DEM line to achieve the desired beam profile at extraction. It became clear that the instrumentation available to the AD team in the DEM line was not sufficient to make this an easy task. Of the two beam monitors installed at the end of the line only one was operational, and as it turns out, since our beam energy is higher by a factor of 10 over normal operation, the sensitivity of the silicon strip detectors was inadequate to produce a signal for the relatively large beam spot we desired. After about 8 hours of running we saw a first indication of extracted beam at the entrance to our phantom using self-developing film (GAF chromic film), which changes color upon irradiation with a given minimum dose. As no other diagnostics on the beam spot shape and size was available, we decided to use the tune settings as of this time and proceed with more dosimetry studies using film. The main purpose of this was to establish the location of the beam focus, the actual shape of the beam spot, and the location of the Bragg peak in comparison to the Monte Carlo calculations.

After the end of these tests we located the biology phantom to match the observed location of the beam spot in preparation for the two shifts on June 9.

While the GAF chromic film shows the effect of irradiation immediately, a proper analysis of the beam profile requires scanning the film in a densitometer. Therefore we could only establish the location of the beam spot, but were unable to get obtain exact information on the spot size. Two problems became apparent in subsequent analysis. As the beam spot was highly elliptical, the sigma in the vertical direction was significantly below the desired value of 0.94 cm, resulting in a higher than desired dose variation in the vertical direction. Additionally, the average sigma of the beam was below 0.94 cm, resulting in an effective dose, which was higher than the expected dose by a factor of nearly two.

For the intensity measurements we had obtained two conflicting numbers. One was a current pick-up at the entrance of the DEM line provided by the AD team, the other was the read-out from a current transformer from Bergoz, which according to factory specifications was absolutely calibrated. These two readings differed by a factor of approximately 1.9. As we had no indication of the quality of the beam transport in the DEM line we were forced to accept the lower one of the two readings (Bergoz BCM), attributing the difference from the AD reading to beam loss in the transfer line.

Using the limited information we had available at the end of the two shifts we recalculated the necessary irradiation time for a variety of doses and concluded that we only could perform a subset of the desired irradiations. Using the intensity measurement from the Bergoz Beam Current Monitor (BCM) and the assumption that the beam spot would match the optics calculations we had performed beforehand, the total of 16 hour of beam time was used to achieve nominal doses of 1, 2.6, 4.6, and 13.6 Gy peak doses.

After the end of the irradiation period the cell samples were transported back to Vancouver, where the 6 mm diameter cylinders of gel containing the cells were extruded and sliced in 1 mm thin slices. For each of these slices a specific number of cells, depending on the axial location in the tube of the specific slice and the predicted survival fraction for this location, was plated and cultured in growth medium. Plating a different number of cells for each slice, depending on the predicted survival values, leads to assuring for each dish a statistical significant number of cells for the counting and thereby enhances the accuracy achievable in the analysis. After an incubation period of 5 - 6 days, the number of surviving cells was determined by counting the number of colonies in the individual dishes.

Only at this moment it became clear that the cells had received a higher dose than predicted and that no surviving cells could be found in the Bragg peak for the three highest doses. In addition, for the highest dose (nominal 13.6 Gy) no surviving cells were found in the plateau region as well. After further studies (see below) we could determine that two effects had combined to result in a significant under estimation of the doses: (1) The factor of 1.9 in difference between the Bergoz BCM and the number of antiprotons reported to be extracted by the AD team could be resolved in favor of the higher number, and (2) the actual beam spot size (available only after a densitometer scan of the GAF film had been completed) indicated a sigma of 0.67 instead of the planned 0.85, resulting in a factor of approximately 1.6 higher fluence in the sample tube. A later detailed recalibration and analysis of the dose measurements resulted in the corrected doses of 3.4, 7.6, 17.5, and 40.7 Gy.

Aside from these problems, this first experiment, which was designed as a test and preparation experiment, was successful in producing a set of important information:

- The method as such worked very well. We were able to deliver a significant dose of radiation to four different cell samples in a period of about 10 hours of net beam time.
- We were able to culture and prepare large numbers of cells at the University Hospital in Geneva (HUG), expose them at the AD, and transport these cell samples back to Vancouver for analysis and could extract good data in the plateau from three of the samples and obtained a complete vs. depth response for the lowest dose.
- Based upon the plating efficiency, accurately known through our ^{60}Co calibration measurements, and the observation of zero surviving cells in the peak for the medium two doses, we are able to give an upper limit for cell survival in these slices, resulting in the plot shown in figure 3. Here we converted all material intercepting the beam into water equivalent depths.
- Even for the two highest doses, which produced 4 logs of cell killing in the peak and the plateau regions, minimal killing of cells beyond the Bragg peak and in those tubes placed in radial directions could be observed. From this we can conclude that the peripheral damage due to neutrons, pions, and gammas resulting from the antiproton annihilation is minimal, at least for these small irradiation volumes.

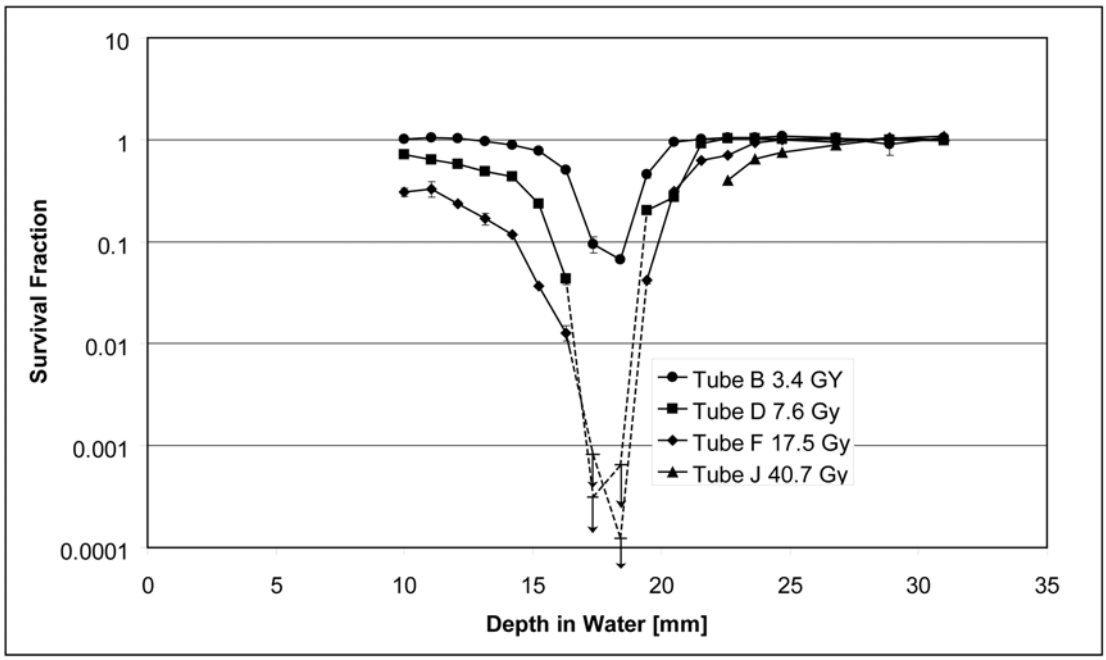

Fig. 3: Survival of cells vs. depth in water for irradiation doses in the peak of approximately 3.4, 7.6, 17.5, and 40.7 Gy. As no surviving cells were found in the slices at 17.5 and 18.5 mm depth we give only an upper limit for the survival fraction in the peak for the 7.6 and 17.5 Gy doses.

Run period B – July 7 – 8, 2003:

After recognizing the significant difficulties in beam monitoring and steering in the DEM line we decided to dedicate the second beam time period entirely to these problems. For this purpose we had requested the installation of a mobile wire chamber after the exit window of the DEM line to give a second diagnostic tool to the AD team for beam steering. We also had contacted A. Mueller and M. Rettig from the Radio Protection Group and had obtained their agreement to assist in an aluminum activation measurement to obtain an absolute value of the total beam intensity delivered over a period of approximately 4 hours. For this measurement we had prepared a stack of 10 aluminum disks of 1.5 mm thickness, which could be positioned in the beam in place of the dosimetry phantom.

Parallel to these efforts on beam steering and fluence measurements we placed LiF thermo luminescent chips (TLD's) of 1.3 x 1.3 x 1 mm^3 in a similar phantom at varying depths in the incoming beam and irradiated them with a prescribed number of antiprotons delivered from the AD. In addition, GAF Film was placed along the beam axis in the phantom to record the dose response of the film.

Even with the improved diagnostics set-up (Mobile wire chamber) it proved more difficult than expected to obtain an antiproton signal at the exit of the DEM line and irradiation experiments could finally begin after about 5 hours of beam tuning. The following 7 hours were used to irradiate a total of 18 TLD chips located at different depths in the phantom with doses varying between 0.75 and 1.5 Gy. As the density of the TLD chips was significantly different from the density of the phantom we avoided stacking TLD's, as this would have made a comparison to Monte Carlo calculations impossible.

After this set of measurements we replaced the phantom with the aluminum stack and irradiated it for about 4 hours (receiving 3.7 x 10^9 antiprotons according to the internal AD pick-up – with the Bergoz BCM reading only 1.7 x 10^9.) Off line analysis of the aluminum stack decided this discrepancy clearly in the favor of the AD reading, and subsequently a modification of the mounting of the current transformer brought the Bergoz instrument in full agreement with the AD readings.

Accordingly the TLD irradiations were analyzed using the AD report for the fluence measurement. Figure 4 shows the prediction for physical dose delivered to the phantom using the number of antiprotons reported from the AD pick-up in the DEM line and the actual beam spot size as obtained from a densitometry scan of a GAF Film sheet placed at the entrance of the phantom. (Due to continued difficulties in beam tuning in the DEM line this spot size was highly elliptical). Overlayed without any normalization are the dose readings from the TLD's placed at two positions in the plateau and four positions in the peak. Agreement within the uncertainties in particle fluence in the beam of about 5% between these measurements and the MCNPX predictions were found in the plateau region, while in the peak region an underestimation was observed pointing towards the expected non-linear response of the TLD's to high LET radiation.

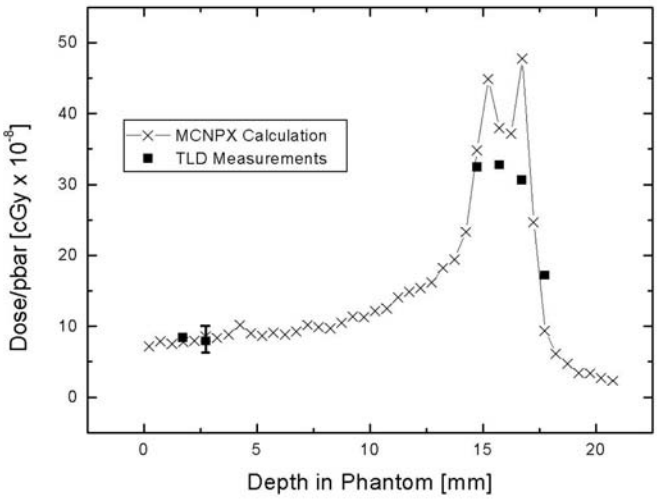

Fig. 4: Comparison between MCNPX calculations of the depth dose profile of antiprotons to direct measurements using thermo-luminescent detector (TLD) chips placed inside a phantom.

To provide a capability for online monitoring of the beam spot position and profile during the irradiations we have developed a beam monitor based on a thin sheet of scintillator material and a CCD camera. We installed a 400 μm thick BC400 scintillator sheet after the Bergoz current monitor and just before the front face of the target. Light generated in this scintillator when a beam pulse passes through is captured by a large numerical aperture lens and imaged onto a digital imaging system comprising a TE cooled camera head with a Kodak CCD chip with 768x512 pixels of 9 x 9 μm area. Initial estimates of the light yield and solid angle of the system indicated that a shot of 3 x 10^7 antiprotons should produce a signal of a few thousand photons on the CCD. Figure 5 shows the image captured from a single shot from the AD. A line profile through the image is plotted as well and the result of a Gaussian fit is in good agreement with the measurements obtained with the mobile wire chamber at the start of the run.

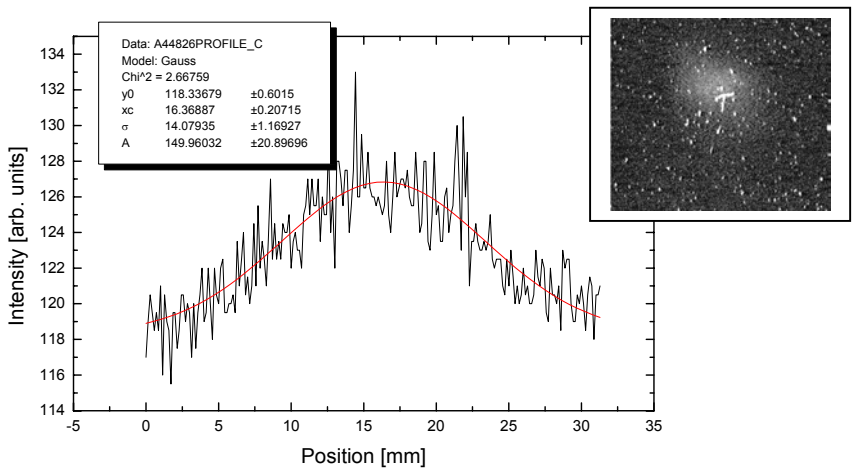

Fig. 5: Image obtained from a single shot of 3 x 10^7 antiprotons passing through a thin sheet of scintillator using a CCD camera. The fit to a line profile taken through the center of the spot shows the width of the beam in excellent agreement with the film measurement of figure 2.

Run period C – September 8 - 9, 2003:

Significant progress in the control of the antiproton beam entering our biological target has been made during the previous run periods. Having achieved both a good understanding of beam tuning and a complete calibration of the fluence measurement we were now prepared for a complete set of biological irradiations.

Based upon the ideal beam spot size of $\sigma = 0.94$ (which we had achieved during a short MD period a few weeks before), and an expected intensity of the AD shots of 3×10^7 antiprotons per spill at 86 seconds time intervals we planned a complete set of irradiation sample tubes, covering doses of 1, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 20 Gy to take about 16 hours of running. At start-up of the machine it turned out that the emittance of the AD beam was larger than normal due to non-optimal performance of the electron cooling. Consequently it was not possible to achieve the ideal beam spot and we started our irradiation program at about noon on Monday with a beam spot with $\sigma = 1.25$. While this was beneficial in terms of radial dose variation, it forced us to reduce the original irradiation schedule to 1, 2, 3, 5, and 24 Gy (the high doses are required to have samples with significant cell kill in the plateau region to extract a plateau survival vs. dose curve).

All six sample tubes (we repeated the 1 Gy dose as a check measurement at the end) were transported back to Vancouver for analysis. Preliminary indications from the stained colonies showed that this set of experiment was successful and that the dose predictions were accurate enough to obtain appropriate colony numbers in the culture dishes. As the number of dishes and colonies in the experiment is much larger than in the test run, the analysis is much more labor intensive and therefore the full analysis is not yet completed at the time of writing of this report. We assume that a full analysis will be available at the time of presenting this report to the SPSC on October 28.

Future work in 2003:

A full radiobiological experiment, while desirable and necessary in the longer run, takes a significant investment in time and money, and as past experience shows, cannot be performed in parallel with other physical studies. Preparation of biological cell samples takes about one full week of intensive work at the Geneva Hospital and requires several key people in the collaboration from different institutes to be present for the entire time. After the actual irradiation the samples currently have to be transported to Vancouver for analysis, which takes several weeks of staff time for the actual counting (depending on the number of samples and the number of colonies per culture dish) and then additional time for the data analysis and compression. In view of the open questions on beam tuning and monitoring, and not having completed the analysis from the last run, having a complete set of data from the September run would not justify mounting such an effort at this time.

Dealing with biological systems one is also facing the problem of repeatability of the experiment. The experimental parameters of a single experimental run can be very well controlled, and as all cells are treated exactly the same way during preparation, irradiation, and analysis, results from different irradiations within one run can be related to each other with high accuracy. Cells can show different biological responses from one run to the next, as a new batch of cells is being used, or as environmental parameters like temperature during transport, plating efficiency at the

start of the analysis, etc. can vary from run to run. This could generate an unacceptable level of uncertainty in the final analysis of the BEDR reported. Therefore it is absolutely necessary and a widely accepted standard in biological studies to repeat the full biological protocol several times.

While we definitely plan to follow this protocol we have decided that before this can be done a complete and detailed understanding of the physical dose delivered to the cell samples is needed. A number of issues concerning dosimetry have been identified by our collaboration, which need to be addressed before further biological irradiations are performed, and we plan to use the remaining shifts in October to complete this part of the work.

The concept of using the Biological Effective Dose Ratio (BEDR) in principle eliminates the need to have absolute and precise dose measurements along the depth profile available. Dose information is only needed to assure the proper range of radiation doses. But to allow direct comparison with standard studies on cell responses to ionizing radiation it would be advantageous to extract a value for the Relative Biological Efficiency (RBE). For this absolute dosimetry in the peak and plateau regions is necessary.

One of the most critical questions to be answered in terms of future work in this direction is the response of standard dosimeters to high LET antiproton annihilation radiation. As very little is known in this field, this question must be addressed experimentally. Using the MCNPX program package we have performed Monte Carlo simulations of the dose delivered vs. depth in our target material for the specific radial profile, fluence, and energy spread for the actual beam used in the experiment. In figure 4 these Monte Carlo simulations are compared to data obtained using standard LiF Thermo-luminescent Detectors (TLD). While we find good agreement in the plateau region, the measured dose falls short of the calculated values by 30 – 50 % in the peak, as expected for high LET radiation. Having full control over all other parameters (fluence, beam spot, etc.) we now can run a number of test experiments to determine the extent of these non-linearities and to search for ways to overcome these limitations. We have ordered a number of different TLD chips and plan to study not only the total light output of these after irradiation but also to look at the individual wavelength components of the light released during read-out. In addition, using thin TLD tablets (0.25 mm) will allow us to use a stack of them to study the dose variation along the axis in the Bragg peak region in more detail.

Due to the strong non-linear response of cells to ionizing radiation, dose inhomogeneities, both in radial and axial directions, have to be understood very well and controlled sufficiently to allow meaningful experiments on cell survival. Using the same set-up as above we can use a stack of GAF chromic film in place of the thin TLD chips to study radial dose profiles at different depths in the material.

Time permitting; we also plan to run a series of measurement without degrader and to use a collimator system to generate a pencil beam. Using film and TLD's we will study the effect of any pion contamination, which may be introduced into the beam by such a system. If we can show that this contamination is small we consider running some test cases in the future in this configuration. This would allow directly comparing our results to work by others using protons and heavy ions found in the literature.

Independent of these studies to be performed at the AD we plan to perform a direct comparison experiment with protons at the ASTRID facility at the University of Aarhus. We will use the identical degrader, the same beam energy and spot size, the

same biological sample preparation, phantom, analysis, etc. to produce a reference data set for direct comparison of the BEDR values for antiprotons and protons.

In order to match the beam conditions at ASTRID to the AD, a beam of negative hydrogen ions at the appropriate energy will be stripped in the ASTRID ring by shifting the energy of the electron cooler to its maximum. The resulting neutral hydrogen atoms will exit through a gap in one of the bending magnets. They will then pass through a scatter foil in which multiple Coulomb scattering will broaden the beam to the desired σ. Here the neutral particles will be stripped to produce protons, which then exit through a thin stainless steel window. Accounting for the energy loss in the scatter foil and the vacuum window this will result in a proton beam with similar characteristics as for the antiproton beam at the AD.

Furthermore, even though the timing of the beam delivery will be different on the microscopic scale, the total time to deliver the radiation will be similar to account for effects of cell repair, i.e. dose rate and recovery. This experiment is scheduled for the time period between November 2003 and February 2004.

Outlook and plans for 2004:

Based on our initial experiments this year we propose to continue our studies into the next run cycle of the AD. The experimental program we envision can be separated into the following independent blocks:

Further development of dosimetry: Absolute dosimetry in mixed, high LET radiation fields is a field of fundamental interest in the field of hadron therapy. For our specific application, continuation of our dosimetry studies together with Monte Carlo calculations will give us a substantial level of confidence in our depth dose profile, and together with better control of the beam tune, this will allow us to extract a value for the Relative Biological Efficiency (RBE) for antiprotons from the data sets. This result can then be compared to the RBE of a similar Spread-Out-Bragg-Peak of protons.

The discrete time structure of the AD beam with the very high instantaneous rate of 3×10^{14} particles per second make it impossible to use standard ionization chambers as for instance used in Sullivan's experiment [2] to determine the physical dose deposited in the target. Standard thermo-luminescent detectors are known to exhibit non-linear responses to high LET radiation [6]. The same is true for radiation sensitive film. Continuation of detailed studies of dose response of different detector methods is therefore an important part of our proposed studies. In addition to the TLD and film studies already mentioned, we are also considering ultra-thin silicon diodes (which may hold the potential of having a higher saturation threshold due to the much smaller number of electron-hole pairs generated). We have made initial contacts with experts from the Semiconductor Laboratory at the Max Planck Institute in Munich on this topic and hope to be able to test these devices in our beam during the next run period. In addition we continue our search for new methods to tackle this problem.

Improvements of the beam delivery system: As already discussed, the discrete time structure of the antiproton beam makes it difficult to use standard degrader systems to produce a spread-out Bragg peak. To achieve the width of the Bragg peak of 3 mm desired in the original experiment we designed a fixed degrader with three thickness levels between 1 and 2.6 mm. According to our Monte Carlo calculation this produces an axial dose profile with variations of up to 20% peak to peak. To reduce the dose inhomogeneity in the Bragg peak region it would be advantageous to use a narrower Bragg peak, which will then have less dose modulation in axial

direction. As we have now improved our biological analysis technique and are able to use sample slices of only 0.5 mm thickness, this can be done and would only require a slight modification to the existing degrader. As an additional benefit, the peak dose delivered for the same antiproton fluence would increase and more samples could be irradiated in the same amount of beam time.

Additional improvements to the experimental set-up would be desirable in the area of beam tuning. Originally, the DEM line was designed to have two dipole magnets installed, which would allow much better control over the steering of the beam. While we have found a tune for the DEM line, which can provide the desired beam quality, this tune is rather sensitive to the performance of the electron cooler and it is sometimes difficult to account for changes in the AD ring. Part of the problems encountered in this summer come from the fact that no steering in the vertical plane is available in the actual DEM line and the AD team has to use elements further upstream. This, together with the fact that the current tune essentially leads to a situation where the beam completely misses the only wire chamber installed in the DEM line, makes tuning up the beam line at the start of an experiment sometimes rather difficult and time consuming. Having additional steering available at the end of the beam line would vastly improve this situation. It is our understanding that the hardware for such an installation exists and, if this actually is the case, we propose to collaborate with the AD team in adding these dipoles to the line during the shutdown this winter.

We have continued our detailed calculations for the tune of the DEM line and have found settings which should allow us to reach smaller beam spot sizes, desirable for some of the dosimetry studies and possibly also for biological studies using different biological protocols where a pencil beam can be used. For beam spots below five millimeter in radius these tunes require changes in lines further upstream, i.e. the 7000 line. We are continuing these studies to search for possible tunes using higher currents in the final quadrupole magnets in the DEM line. This may require an upgrade of the magnet power supplies, which was discussed initially, but then not performed when we found an acceptable beam tune with the existing power supplies. As it turned out, this tune was only valid under optimum electron cooling conditions and was only met a few times. A tighter spot (within the limitations of the radial dose inhomogeneity) would result in shorter irradiation times and would significantly improve the experimental performance.

At the same time we would request a complete survey to be done for the DEM line. Currently only the remnant of some markings of the original survey exist in the zone and these markings are not in agreement with the actual location of the vacuum chamber and exit window. In the first test experiments we established a misalignment of about 1 cm in horizontal direction using GAF chromic film. Even though we now have a direct online beam position monitor available, a proper survey would facilitate changing between dosimetry and biology phantoms.

All these modifications would also be of benefit to other potential users of the DEM line.

Continuation of clonogenic assays on live cells: As indicated in this document, due to problems in beam tuning and dosimetry we were unable to complete the proposed set of biological experiments. While the data we have obtained indicate that this application may hold significant promises, we need to repeat the full irradiation sequence at least once. As we are using biological systems in our measurement we are potentially sensitive to differences due to using a different population of cells, to different temperature variations during preparation, irradiation,

and transport to analysis, varying plating efficiency in the analysis process, and many other uncertainties. Only repeating the experiment will allow us to draw a solid conclusion on the BEDR for antiprotons, which we can then compare to our proton measurements at Aarhus.

As described below, we plan to modify our degrader design to assure a better homogeneity of the dose delivered to the target, both in radial and axial direction. With this new set-up we would then run a second (more complete) set of irradiations using these new beam parameters.

Alternative biological end points and protocols: We have initiated discussions within the collaboration on possibilities to use different biological protocols for our studies which may allow us to perform shorter measurement cycles with a quicker response time to schedule changes and individual shifts becoming available due to unforeseen problems in other experiments. We are engaged in ongoing discussions with interested experts in the field at the Geneva Hospital on this question.

Summary and requests:

Our initial experiments on the biological effects of antiproton annihilation have shown that the method originally proposed by us is viable and will deliver statistically sound data. We have performed two independent sets of biological irradiations, one initial test experiment to establish the proper dose range and one more or less complete set of cell irradiations, which has yielded results for the entire spectrum of relevant doses. We already know that the results from latter experiment are of good quality, the data set is still being analyzed at the time of writing this report.

We have not been able to complete the entire program proposed, partially due to difficulties in beam steering and monitoring, and have invested a significant portion of the allocated beam time to the development of new beam profile monitor and to the calibration of the fluence measurements available to us, both from our own experiment and from the AD operations team.

Based on this we propose to continue our experiment in the next run cycle of the AD and request specifically:

- 4 sets of three shifts (24 hour periods) for clonogenic assays. These will be used to complete one repeat experiment with the current set-up and to perform a set of three runs with an improved SOBP.
- 8 individual shifts to be interspersed between the biological experiments for test experiments on beam monitoring and dosimetry.
- Upgrading the DEM line by installing additional dipoles and upgrading the power supplies for the existing quadrupoles to provide better steering and a tighter focus of the beam.
- A new survey of the DEM line to be performed during the shut down period, when access to the quadrupole magnets, which serve as reference for the beam axis, is available.

We thank the AD operations team for their assistance in setting up the experiment and for providing the best beam tune possible under the circumstances.

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