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USE OF IN-HOUSE DEVELOPED IMAGING SOFTWARE *SCGE-Pro* FOR COMET ASSAY TO QUANTIFY DNA STRAND BREAKS IN MOUSE LEUKOCYTES

R.C. Chaubey, H.N. Bhilwade, V. Sonawane, R. Rajagopalan, N. Joshi and K.P. Mishra
Radiation Biology and Health Sciences Division

and

P. Singh, S.K.Gupta and S.Kailas
Nuclear Physics Division

Introduction

In a collaborative project with Computer Division on development of imaging software for cytogenetic (Chaubey et al., 1999) and DNA damage analysis, we have developed a dedicated imaging software, *SCGE-Pro* for single cell gel electrophoresis or Comet assay (Chaubey et al., 2000). The Comet assay is a powerful tool to investigate genotoxic effects of low and high LET radiations, drugs, chemical mutagens and carcinogens present in the human environment. This assay can be used to detect DNA single strand breaks (ssb); alkali-lab-ile sites and incomplete DNA repair sites, under alkaline condition and DNA double strand breaks (dsb) under neutral condition (Singh, 1996; Chaubey et al., 2001). DNA base damage can be detected and quantified by using endonuclease III and

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formamidopyrimidine-glycosylase (fpg) (Tice, 1995; Pouget et al., 1999) and UV-induced pyrimidine dimers by using T4 endonuclease V (Sauvaigo et al., 1998). This assay has been combined with fluorescence *in-situ* hybridization (FISH) to measure gene specific repair in relation to total DNA or loss of heterozygosity (LOH) for single gene (Collins, 2001). It provides a unique opportunity to investigate intercellular differences in DNA damage and repair kinetics in any eukaryotic cell.

A Folded Tandem Ion Accelerator (FOTIA) has been commissioned at Nuclear Physics Division, BARC (Singh, 2001) and very recently it was possible to obtain proton beams in air, whereby it was possible to conduct studies on cellular systems. Use of protons and other charged particles in radiotherapy is known since long time. Due to Bragg peak effect and sharply defined range, protons offer the potential to confine the major energy transfer to the tumor mass and thereby minimizing damage to the adjoining normal tissues. (Wieszczycka and Scharf, 2001). We have carried out preliminary studies to investigate the effect of 3.3 MeV proton beams on DNA damage on mouse peripheral blood leukocytes using Neutral Comet assay. Images of the comets were acquired using a Digital Imaging System and analyzed using the software, *SCGE-Pro*, developed for Comet assay. Fig.1 shows the monogram of the software *SCGE-Pro.*

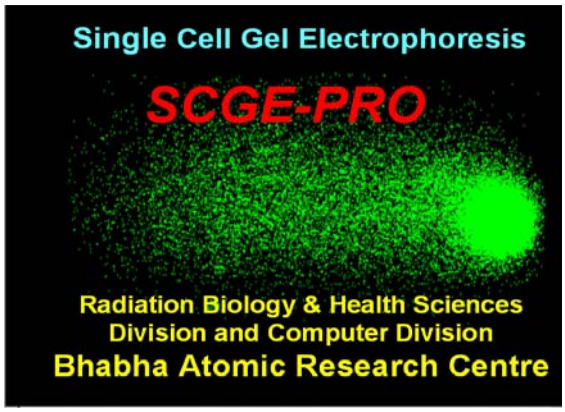

*Fig.1: Monogram of the software **SCGE-Pro** for Comet Assay*

Single Cell Gel Electrophoresis or Comet Assay

- In the comet assay the cells are suspended in agarose, the gels are made on microscope slides, lysed with any detergent or high salt solution, and the liberated DNA is electrophorased under alkaline or neutral condition.
- DNA is negatively charged. Depending on the size and total negative charge, the DNA fragments migrate to different distances towards anode. After electrophoresis, the cells are stained with any DNA specific dye and observed under a fluorescence microscope. The cells appear as comets with brightly fluorescing nucleus and diminishing fluorescence intensity in tail (Fig.4).
- We have developed an imaging software, *SCGE-Pro* for comet assay to measure various comet characteristics e.g. tail moment (TM: product of fraction of DNA in the tail and tail length), tail length (TL), percent DNA in the tail (%DNA-T) or head (%DNA-H), which are considered to be more consistent and reliable parameters of DNA damage.
- The data is automatically stored in the application specific format in the result file, which can be imported to Microcal Origin ver. 5 for various statistical calculations and graphical representations (Chaubey et al., 2001).

Materials and Methods

Preparation of microgel slides for proton irradiation

Mouse blood (10µl) was mixed with 100µl low melting point agarose (0.75%) at 38°C and layered on fully frosted slides pre-coated with 200µl of high melting point agarose (0.5%) and preserved on ice. The slides were held vertically in the path of proton beam in a holder with ice packing on the reverse side of the slide for different time intervals.

Irradiation

Folded Tandem Ion Accelerator (FOTIA) at BARC

Proton beams were accelerated using FOTIA facility at BARC (Singh, 2001 & 2002). Proton beams were taken out in air by using a 20µ Titanium foil as vacuum barrier (Fig. 2). During these experiments, the average current was 2 nA (1.3 x 10^{10} protons/sec) with a beam size of about 4 mm on the sample. In FOTIA, the negative ion beams extracted from the SNICS-II source are pre-accelerated up to 150 keV. Negative ions of the desired mass are selected using a 70° -dipole magnet (ME/q^2=12, R=40 cm) and injected into the low energy accelerating tube through a 20° electrostatic deflector. An electrostatic quadrupole triplet is used to focus and match the beam parameters to the acceptance of the low energy tube. The electrons of these accelerated negative ions get stripped off at the stripper and charge state of the positive ions thus produced is selected with the 180° magnet (ME/q^2=10, R=30.5 cm) inside the high voltage terminal before being bent into the high energy accelerating tube where they further accelerated.

At the exit of the 180° magnet, the beam diverges. An electrostatic quadrupole doublet is used to focus the beam before it enters the high energy tube. The beams accelerated in the high energy accelerating tube are focused using a magnetic quadrupole triplet before being analyzed by the 90° magnet. The high-energy beam is analyzed by the 90° dipole magnet designed for ME/q^2=50 and R=75 cm.The beam is transported to the experimental area using a magnetic quadrupole triplet and a switching magnet. Beam profile monitors and Faraday cups have been installed in the beam line to measure the size, shape and position of the beam.

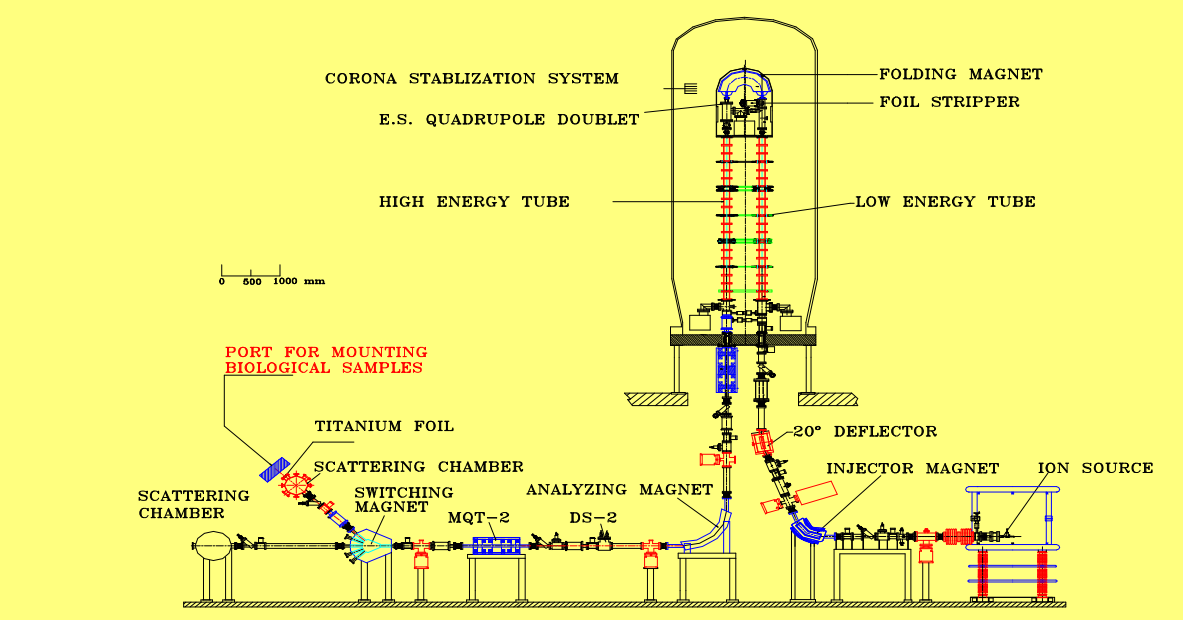

Fig. 2: Schematic diagram of the 6 MV Folded Tandem Ion Accelerator

Table 1: The final beam energies at a terminal voltage of 6 MV for ions with charge state q.

Ion	Z	q	Relative %	E_f
^{1}H	1	1	100	12
^{2}He	2	2	100	18
^{12}C	6	4	52	30
^{16}O	8	5	47	36
^{24}Mg	12	6	39	42
^{28}Si	14	6	34	42
^{32}S	16	7	34	48
^{37}Cl	17	7	33	48
^{40}Ca	20	7 8 9	31 26 12	48 54 60

The FOTIA is an electrostatic accelerator with a maximum terminal voltage of 6 MV. For a terminal voltage of V_T the final energy (E_f) of the beam is given by the relation $E_f = E_i + (1+q)\, eV_T$, where E_i is the energy of the beam at the exit of the ion source and q is the charge state of the analyzed ions. The FOTIA (Singh et al., 2002) has capability to deliver accelerated beams of up to A= 40 and maximum energy of 60 MeV for a charge state of 9^+ at 6 MV (Table 1).

Sample Irradiation: Whole blood samples from mouse were embedded in agarose on fully frosted slides and exposed to 3.3 MeV protons, 2 nA in air for different times e.g. 0, 1, 2, 4, 6 and 8 minutes at 0-4 °C.

DNA double strand breaks using neutral Comet assay

After irradiation, the slides were kept in the lysing solution containing 2.5 M NaCl, 100 mM EDTA, 10% DMSO, 1% sodium N-lauroylsarcosinate, 0.5% triton X-100, 10 mM Tris-HCl pH 10 for 1h at 4-7 °C. The slides were then washed three times with 300mM sodium acetate 100 mM Tris-HCl, buffer pH 8.3 and equilibrated in the same buffer for 1h at 4-7 °C. The slides were transferred to the electrophoresis apparatus and were electrophorased in the same buffer at 10 °C for 1h at 10 volts and 14 mA. The slides were rinsed with distilled water and stained with 50 µl SYBR Green I (1:10,000 American Bioprobes).

Digital Imaging System

- A fluorescence microscope with epi-fluorescence facility with HBO 50 high-pressure mercury lamp, (Carl Zeiss, Leica or equivalent).
- 0.5x camera adapter lens, high performance color camera with 750 lines horizontal resolution (KY - F55BE 3CCD, JVC, Japan, or high resolution digital camera).
- The Integral Flashpoint Intrigue frame grabber used in this system is a PC based card and it accepts color composite video output of the camera. It digitizes each of RGB planes at a tonal resolution of 24 bits per pixel. It has spatial resolution of 768 x 576 per frame.
- A Pentium P-III computer with super VGA color monitor, CD-ROM drive, 20 GB Hard disk, CD writer for image storage, and HP Desk Jet printer.
- Cost of complete imaging system with the software including Camera microscope, Frame grabber and PC with accessories: Rs 6 – 8 lacs.

Steps involved in measurement of DNA damage using the SCGE-Pro system

The images of the comets are observed at 40x magnification using a fluorescence microscope with Filter 15 (BP546/12, FT580, LP590) of Carl Zeiss. The images of the individual comets are captured using a 3-CCD camera and stored in separate files. The total SYBR Green I fluorescence intensity was taken as total DNA content in the comet. The software allows quantitative measurements of total fluorescence of the comet, fluorescence of the tail (%DNA-T), length of migrated DNA fragments (TL) and it finally calculates the tail moment (TM, product of fraction of DNA in the tail and tail length) for comparing the DNA damage (Chaubey et al., 2000)

Results and Discussion

Present studies were undertaken to investigate the effect of proton beams on DNA damage in mouse leukocytes using comet assay. Most of the biological effects of protons are produced due to primary ionization and production of secondary electrons in the path it traverses. When the proton beam passes through the cell it causes excitations and ionizations of atoms and molecules and thereby leading to chemical changes in biomolecules at cellular and sub-cellular level and ultimately leading to mutation, transformation and cell death (Wieszczycka & Scharf, 2001). Based on experiments carried out in the Spread Out Bragg's Peak (SOBP), the relative biological efficiency (RBE) of protons has been found to be 1.1. In fact the RBE of protons has been shown to increase with the decrease in energy. Several sensitive techniques e.g. pulsed field gel electrophoresis (Weber and Flentje, 1993), alkaline or neutral filter elution (Heilmann et al., 1993; Eguchi et al., 1987), alkaline unwinding (Heilmann et al., 1993), sucrose density gradient centrifugation (Roots et al., 1979) have been used to detect DNA damage induced by high LET radiations. Measurement of DNA damage induced by protons or heavy ions at single cell level will be more appropriate system to study cellular damage. We have used this assay to assess the effect of proton fluence on DNA damage at single cell level.

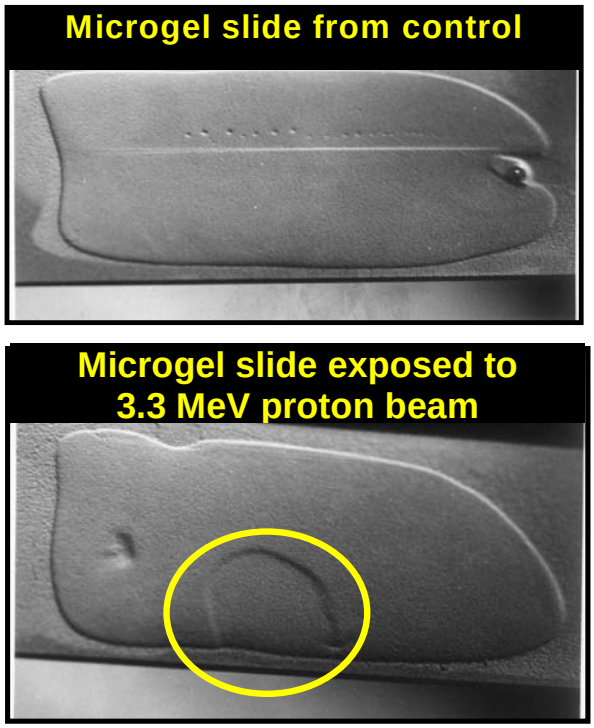

Fig. 3: Microgel slides with cells from control and post proton irradiation

Fig.3 shows the microgel slides from control and post proton irradiation. As can be seen from the figure, a distinct area of irradiation can be seen on the slide. Fig. 4 shows nuclei from control and proton beam irradiated cells. The figure also shows nuclei with different extent of DNA damage and various steps involved in measurements of comet characteristics using the software *SCGE-Pro.*

Fig. 5 shows the distribution of cells with different extent of DNA damage following proton beam irradiation. As can be seen from the figure, heterogeneity in response of cells to proton beam is seen. This could be mainly due to the differential radiosensitivity of mouse leukocytes to radiation. Particle irradiation is heterogeneous in nature and some of the cells may be hit by one, two or more particles. Figure 6 shows the dose-response effect of proton beams irradiation on mouse leukocytes. A dose dependent increase in %DNA -T, TL, and TM was seen with

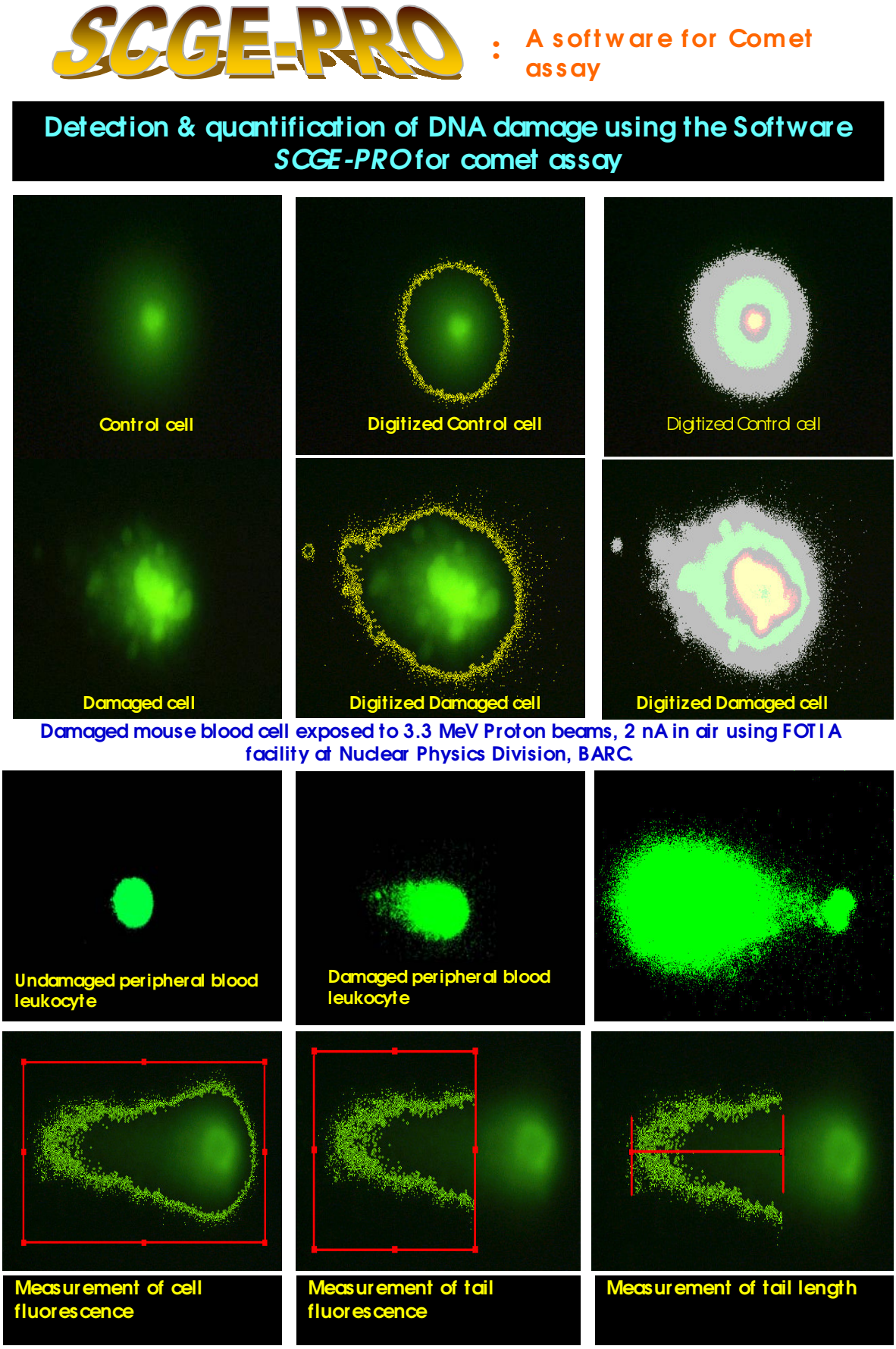

Fig. 4: Comets from control and proton irradiated cells and steps involved in measurements of comet characteristics using the software SCGE-PRO

Proton Energy: 3.3 MeV, Fluence: 1.0 X 10^{11} particles/sec/ cm^2, Current: 2 nA

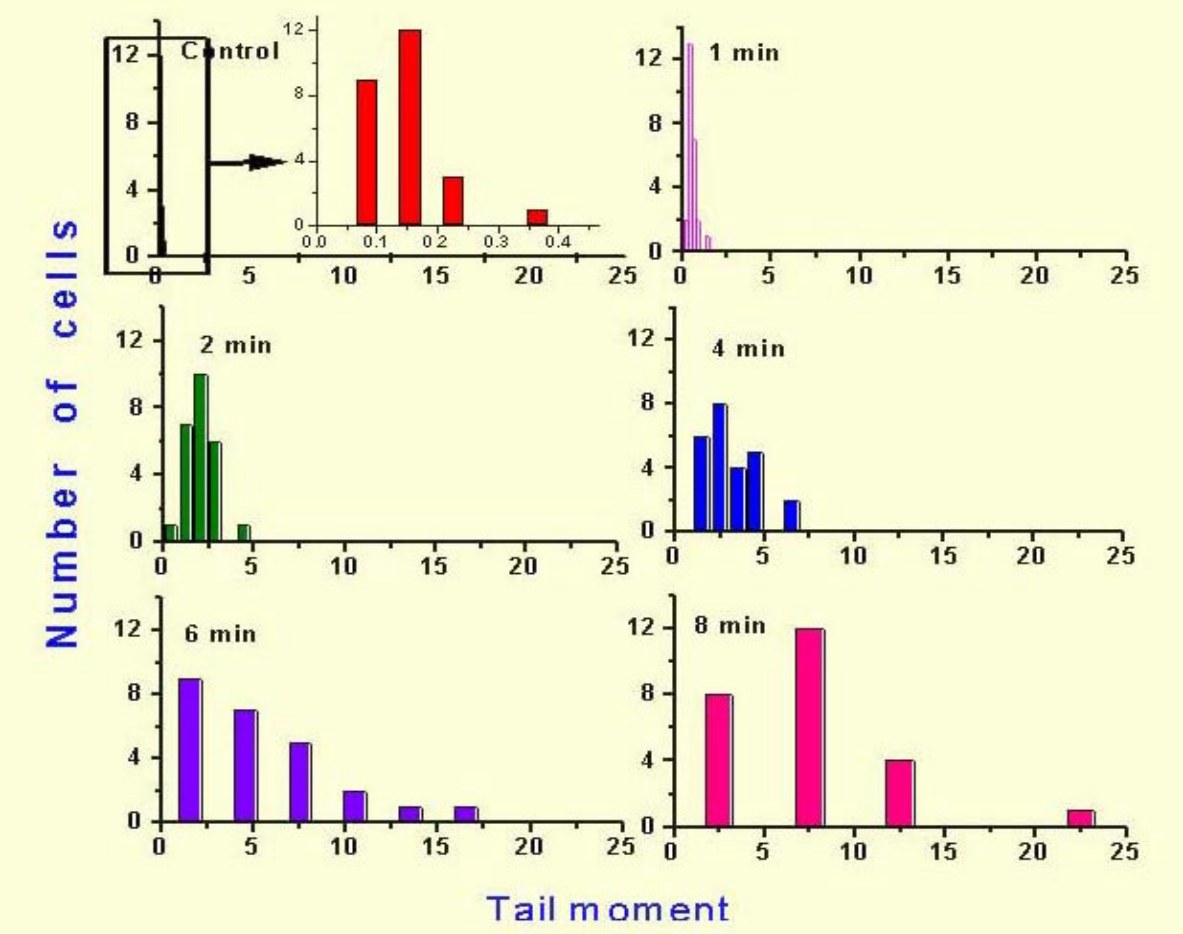

Fig. 5 Frequency distribution of tail moments of comets induced by proton irradiation in mouse blood leukocytes

Proton Energy: 3.3 MeV, Fluence: 1.0 X 10^{11} particles/sec/ cm^2, Current: 2 nA

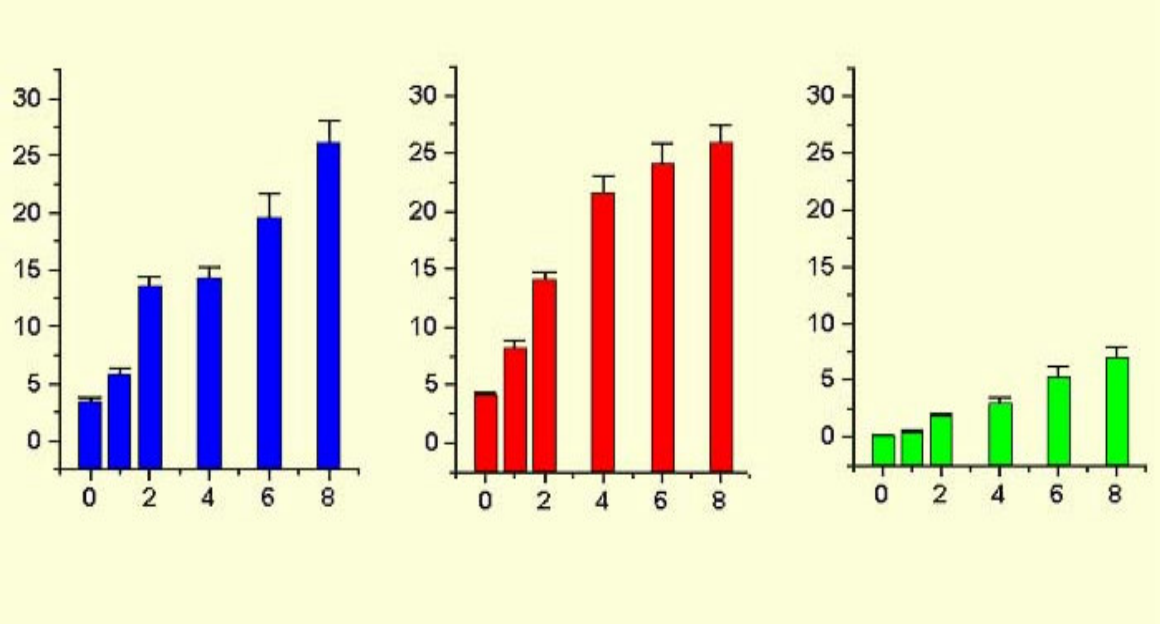

Fig. 6: Effect of proton irradiation for different times on DNA in tail, tail length and tail moment of mouse blood leukocytes

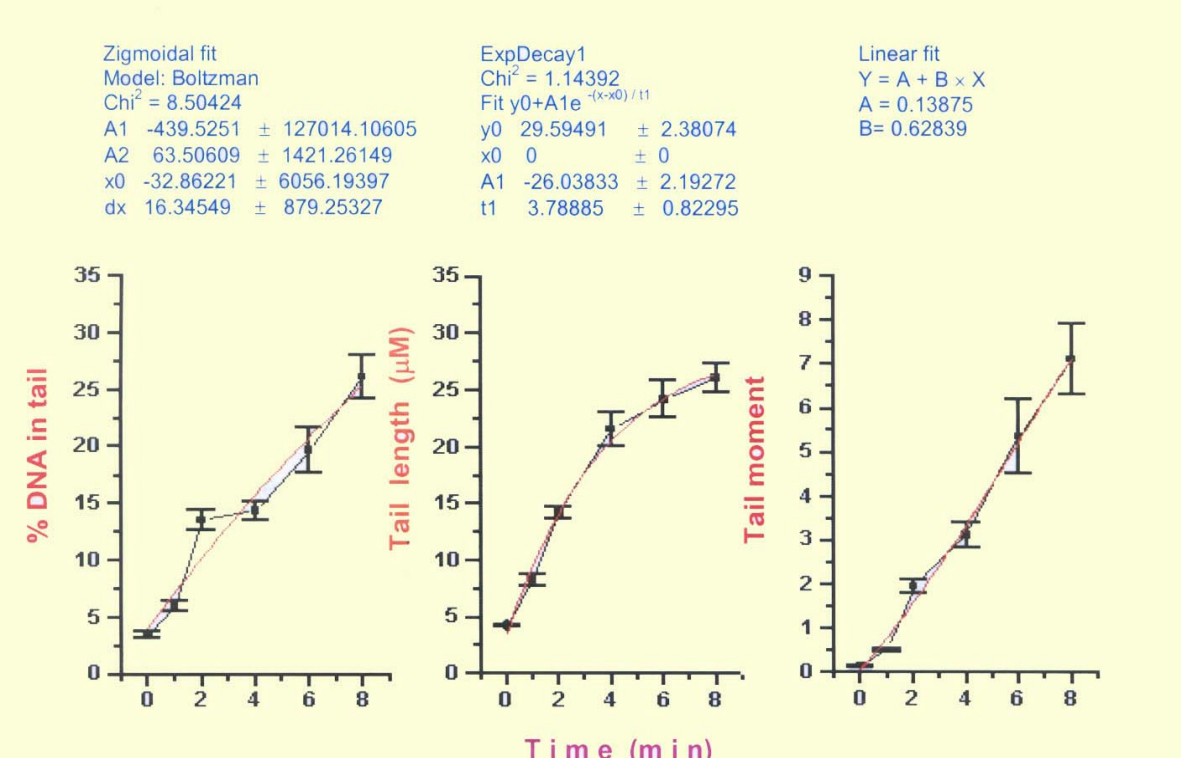

Fig. 7: Proton induced DNA double strand breaks in mouse blood leukocytes: statistical analysis

increasing duration of proton beams irradiation. A statistically significant increase in DNA double strand breaks (dsbs), as indicated by increase in %DNA-T, TL and TM, was observed at all the time intervals. Fig. 7 shows the statistical characterization of the dose response curve with different parameters of DNA damage e.g. %DNA-T, TL and TM after proton beams irradiation. A statistically significant increase in DNA double strand breaks was observed with increase in duration of exposure. It has been reported in literature that DNA double strand breaks increase exponentially in relation to particle fluence i.e. number of particles per unit area (Testard and Sabatier, 2000). Our present data on DNA damage with different duration of exposure to proton beams also show exponential relationship as the best fit (Fig.7). It has been shown that the RBE for DNA dsbs after proton irradiation is 1.2 at 5 keV/μm that rises to about 2.5 at 70 keV/μm (Friedland et al., 2002). Belli et al., (2001) have investigated the effect of low energy protons having LET values ranging from 11 to 31 keV/μm i.e. energy in the range characteristics of the Bragg's peak using the sedimentation technique on DNA double strand break production and rejoining in V79 cells. They have observed that the initial yield of dsb is quite insensitive to proton LET. Their results also indicate that dsbs are not homogeneous with respect to repair and give support to the hypothesis that increasing LET

Applications of Comet Assay

- **Clinical: Prenatal diagnosis, DNA repair deficiency syndrome, Diabetes, Cancer Susceptibility, genomic instability in disease state.**
- **Human biomonitoring: Aging, Nutrition**
- **Environmental biomonitoring – aquatic / terrestrial conditions**
- **Genotoxicity evaluation of radiation & chemicals in human & animal models**
- **Radiation biology**
- **Free radical biology**
- **Clinical and molecular epidemiology**
- **Agricultural Sciences**

leads to an increase in the complexity of DNA lesions with a consequent decrease in their repairability. One of the significant observations during the present study was the presence of highly damaged cells (HDC), characterized by presence of large DNA fragments in clusters. Besides a large number of apoptotic cells were also observed. Further study with protons on DNA damage and repair in normal and other malignant cell lines is in progress.

The software is available for sale to National and International Users on payment basis. For further details, contact Dr R.C. Chaubey, E-mail: rchaubey@apsara.barc.ernet.in, or Mr V.K. Chadda, E-mail : vkchadda@apsara.barc.ernet.in Tel. No. (022) 25593949; Fax No. (91) (22) 25505151/25519613.

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CALCIUM SUPPLEMENTATION : TRUTH AND MYTH

D.N. Pahuja and M.G.R. Rajan
Laboratory Nuclear Medicine Section,
Radiochemistry & Isotope Group, BARC.

Increased Calcium(Ca) intake (at 1.5g/day; average Indian diet <1.0g/day) has been shown to help in strengthening the bone by curtailing bone-resorbing activity (a major cause for the induction of Osteoporosis) in the elderly population (>60y, particularly females). This is indicated by the reduced levels of the sensitive bone-resorptive markers, particularly parathyroid hormone (PTH), hydroxy proline/creatinine ratio and dipyrolidine & pyrolidine/creatinine ratio. A high calcium intake is also demonstrated to reverse the secondary hyper-parathyroidism and increased bone resorption, resulting in the significant reduction in the incidence of fractures in elderly population (1,2). The normalizing influence of extra Ca-supplementation on bone-seeking radio-pharmaceutical, ^{99m}Tc-MDP, used in the diagnosis of bone disorders; as well as Ca, modulating the propensity of ^{99m} Tc-Phytate uptake by bone and liver, further suggests that Ca prevents undue mobilization of bone which may otherwise lead to the onset of osteoporosis (3,4).

Due to the lack of adequate Ca in our regular Indian diet, it is necessary that we must supplement ourselves regularly with extra Ca (1.0–1.5g/d) and adequate exercise. This extra Ca-intake must not be considered as a prescribed medicine but the necessary dietary requirement like other nutrients: vegetables, fruits etc., which must be taken for the necessary maintenance of our good health.

However, there has been a strong misconception, even in the medical fraternity, that extra Ca-intake leads to formation of kidney stones. In fact, it is the extra Ca, which would help in preventing stone formation by chelating oxalates/citrates (main culprits) present in our food, to insoluble complexes, which are then excreted from the GI tract. Here, it is important to understand that only 20–30% out of ~1000mg/d of Ca ingested, gets absorbed and the rest is utilized for reacting with the unwanted products of the digestive process like oxalates, citrates, bile salts, fatty acids forming insoluble complexes, which are then excreted out in the faeces. Inadequacy of dietary Ca will allow the soluble oxalates and citrates present in our food to escape from getting complexed with Ca in the gut. They are transported to the kidneys by the blood and get insolubilized there with Ca. (Ca comes to kidney during its normal metabolic pathway where most of it is reabsorbed). The insoluble Ca oxalates/citrates deposit in the kidney and form the nucleus on which further deposition of Ca oxalates/citrates takes place over a period of time to form kidney-stones (5).

Colon cancer in the susceptible individuals could also be viewed as an unfortunate consequence of inadequate dietary Ca. On high Ca-diet, ~76-80 % of the ingested Ca remains unabsorbed in the intestinal lumen where it forms insoluble complexes with bile acids and unmetabolized fatty acids, thus sparing colonic mucosal membrane of unnecessary irritation due to these compounds. The insoluble complexes of these compounds with Ca are excreted. In normal course, to completely complex the irritant-residues from fats in a typical diet, one would require unabsorbed Ca of ~600–800mg/day – a value requiring an ingested Ca of at least ~1200mg/day. In fact, in quite a few cases of colon cancer, Ca is reported to being used successfully as a chemo-preventive agent to control the proliferation of colon cancer. In most

of these cell lines, [^{3}H]-thymidine–uptake, an indicator of cellular proliferation, is lowered in the presence of Ca (5).

An inverse association between dietary Ca intake and blood pressure is seen in many adults. The hypertensive subjects had significantly lower serum levels of ionized Ca. In another study, it has been noted that the subjects consuming high Ca diet had lowered systolic pressure. In one of the animal experiments, it was observed that rats maintained on lower Ca-diet for 7 weeks, compared to controls, had reduced gain in their body weight and raised blood pressure, consequent to reduction in the circulating levels of Ca. This could be reversed by putting these rats back to normal Ca-diet (5,8).

In another set of experiments, a group of rats rendered hypocalcemic due to Vitamin D deficiency, were found to have reduced intestinal Ca-transport and retarded growth. Laboratory tests showed that these hypocalcemic rats had altered hepatic function, raised levels of the hepatic enzymes and decreased plasma protein synthesis, and histopathology of the liver was suggestive of periportal necrosis (6). Hypocalcemia also resulted in the significant reduction of hepatic antioxidant enzymes – Super Oxide Dismutase (SOD) and Glutathione Peroxidase (GP), which are responsible for scavenging the free radicals, while lipid peroxidation was enhanced. Extra Ca-supplementation to these hypocalcemic animals, could normalize most of the parameters of the liver function as well as both the important anti-oxidant enzymes (SOD & GP). This is due to the influence of Ca on the stability and maintenance of Vitamin E, Glutathione & Protein–thiols, the major players in scavenging free radicals (7). These observations clearly indicate the ubiquitous nature of Ca, which is also important for many other cellular and intracellular functions besides its effect on bone strength (8).

Reduced Ca-intake evokes the response for Ca-conservation through the interplay of the Calciotropic hormone axis: Parathyroid hormone-Vitamin D-Cacitonin. When this is inadequate, Ca deficiency results. The hormone axis controls the hierarchy of regulation–levels, mentioned below (8):

1. Body Ca, i.e. skeleton, is at the bottom of this hierarchy and most vulnerable to the state of Ca-deficiency.
2. Next is the concentration of Ca in the Extra Cellular Fluid (ECF), which is a well governed area of Parathyroid hormone – Vitamin D endocrine system. It is because the ECF-Ca must be conserved even at the expense of bone that bone is at the first level to be sacrificed.
3. The third level is at the boundary between the ECF and the interior of the cell.
4. Analogous boundary also exists between intra cellular Ca–concentrating membranes and the cytosol where Ca–channels and other transport systems are operative.
5. Last, and apparently most tightly conserved Ca, is at the molecular level where Ca is bound to calmodulin and other proteins present in the cell and vascular membranes.

However, the clinical manifestations of the cellular dysfunction related to impaired cellular Ca metabolism would exhibit several features as mentioned below:

1. First - the emergence of the clinical disorders would occur in later life, as multiple compensatory mechanisms gradually fail over protracted periods of time.
2. Second - the disorders, that would develop might be anticipated to reflect the hierarchy of cell function, i.e., disorders related to cell division and growth or energy metabolism should pre-dominate.
3. Third - since multiple cell types would be affected over time, there may be strong concordance of multiple medical disorders.

This is clear from the fact that as the age advances, the degree of Ca-deficiency gradually increases more due to inadequate intake of Ca

coupled with the reduced Ca-absorption. This leads to increased secretion of PTH, and consequently, the bone loses Ca due to increased bone resorption, leading to the debilitating bone-disease, *osteoporosis*. This is seen to be associated with the rise in the vascular Ca due to the blunting of the Ca-concentration gradient, increasing the chances of arteriosclerosis. All this would also depend upon the contribution of the genetic influences and environmental factors, which would exhibit differing sensitivities to the exposure to Ca-deficiency (8).

There are several chronic disorders linked to the inadequate Ca-intake, which we need to be informed about. Besides osteoporosis, the inability of the cells to regulate their own growth and replication could contribute to the development of both cancer and cardiovascular diseases resulting from the vascular hypertrophy and the change in the peripheral resistance. Several systemic metabolic disorders may be initiated due to lack of adequate Ca, affecting normal cellular metabolism. This impairment could contribute to the development of the hyper lipidemic state (5,8).

Influence on the cell membrane integrity, interfering with the Ca dependent transduction of the hormone receptor-signal, would impair the normal target organ response to hormonal action such as failure of insulin to regulate normal cellular glucose uptake, as in type-II diabetes. In this regard, lower bone mineral density (BMD) and blunted response of PTH and Calcitriol to hypocalcemia has been noted in diabetes (5,8).

All this boils down to the fact that adequate Ca-intake is a must for the maintenance of our normal health, right from the young age, failing which it could lead to various deficiency disorders. This provides us an important food for thought to reassess our dietary habits with regard to our Ca–intake. It is true that agricultural revolution has caused substantial modifications in our dietary habits and the mother nature has been kind enough, trying its level best to adjust and reorient our body to the altered environmental conditions. However, the natural adaptation of the cellular milieu seems to be still inadequate to maintain proper balance. It must be appreciated that agricultural revolution is a relatively recent event on the time scale of evolution and it is highly likely that human genome would not have sufficient time to adapt fully to a change of this magnitude due to rapid industrialization. In this regard, if we compare our dietary intake with that of our fore- fathers who were hunters and gatherers, it has been noted that their diets were rich in fiber, protein, vitamin C and particularly Ca - estimated to be 4–5 times more than what is available from our present Indian diet - which is rich in salt and fat but hardly provides 300 – 500mg Ca/day, when minimum requirement of Ca is 1g/d. Even, NIH/WHO consensus has recommended our Ca–intake must be raised from 1g/d to 1.5 to 2.4g/d, depending upon the age and physiological conditions (9,10).

CLINICAL FEATURES OBSERVED IN HYPOCALCEMIA

HYPOCALCEMIA	CLINICAL SYMPTOMS
MILD	No symptoms or tingling and numbness in the fingers and toes. Chvostek's sign if provoked.
MODERATE	Both Chvostek & Trousseau's signs, more on provocation.
SEVERE / ACUTE	Above symptoms plus possibility of Laryngospasm, Bronchospasm and seizures.
CHRONIC	Papiledema and basal ganglia – calcification, besides cataract, dry skin, coarse hair, brittle nails and defective dentition.

RESPONSE OF HYPOCALCEMIC PATIENTS TO THEIR MANAGEMENT WITH EXTRA CALCIUM & VITAMIN D - SUPPLEMENTATION.*

MILD HYPOCALCEMIA	MODERATE HYPOCALCEMIA	SEVERE HYPOCALCEMIA
Become normo-calcemic with extra oral supplementation of Calcium (1.5-2g/d) and Vitamin D (~ 800 I.U. /d)	Become normo-calcemic with extra oral supplementation of Calcium (2.0-3.0g/d) and Vitamin D (5 lacs I.U.) intra-muscularly every 3-4 weeks. Some patients may require i.v. infusion of Calcium.	Difficult to manage only with oral Ca & Vit. D - supple mentation, besides i.v. infusion of Ca. Better manageable with Rocaltrol (0.5-1ì g/d) along with oral Calcium (~1.5–2g/d).

* It is advisable to monitor blood calcium levels every 4–6 weeks in case of severe and moderate hypocalcemia and every 3–6 months in case of mild hypocalcemia.

All this emphasizes the need for all of us to engage in more intensive dialogue with general public at large to educate them and increase their awareness about the serious implications of the inadequate intake of Ca, so that it becomes easy to embark on the road to recovery from the onslaught of many debilitating/chronic diseases. It is advisable to start Ca-supplementation from the early age, more during pregnancy/lactation periods and better to take Ca on empty stomach for its better absorption.

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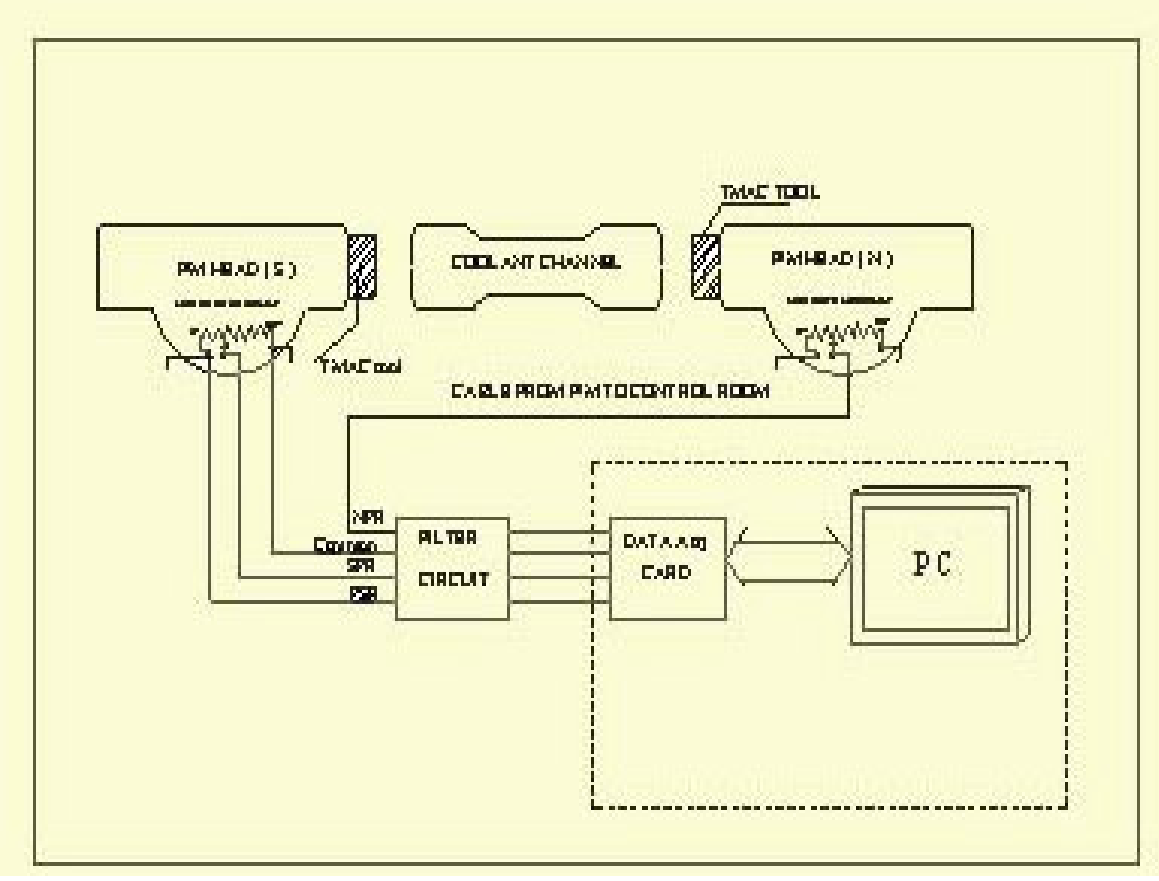
TECHNIQUE FOR MEASURING AXIAL CREEP OF 220 MWe PHWR COOLANT CHANNELS

Axial creep monitoring of the coolant channels is essential to be done during every annual shutdown in Pressurized Heavy Water Reactors. Previously, this measurement was done by optical method, which was manual as well as time and man-rem consuming. In view of this, a technique called “TMAC” (Technique for Measuring Axial Creep) was developed at RTD some time in mid 80s and was implemented in all reactors. The fuelling machines make contact with the help of specially made tool to the coolant channels from both sides and potentiometer readings are taken for measuring the distance. Subsequent measurement in comparison with non-creeping channels of RFT gives the creep since last measurement. The system consists of hardware and software for acquiring the data and processing them to get the result in the desired format. Since inception, the system has been modified as version 2.0.

Need was felt to standardize the system for all the operating stations and also to implement additional features in the software. In view of this, TMAC (ver 3.0) has been developed at RTD. Following are the major features of the system.

Data Acquisition card

Data acquisition card (ADC-528) has been indigenously developed to eliminate its dependency on market. The circuit design was worked out so that user can fabricate the card whenever required or do maintenance.



Block diagram of TMAC

Features of ADC-528 :

- 16 single ended analog input channels
- Input range
  Uni-polar : 0 to 10 V
  Bi-polar : -5 to +5 V
- 12 bit resolution
- 16 Digital input / 16 Digital output
- PC bus (ISA) compatibility

Software

The software, which was developed in C language, was modified such that it takes care of different types of temperature correction factors to be applied on measured creep value for different combinations of pressure tube material and geometrical layout. It was also made compatible to ADC-528.

A window based software CREPT (Creep Report generation Tool) was developed to facilitate report preparation in desired formats. It makes possible to export the data to popular format such as MS EXCEL to facilitate analysis. It also facilitates storing of creep adjustment details and report preparation.

User's Manual

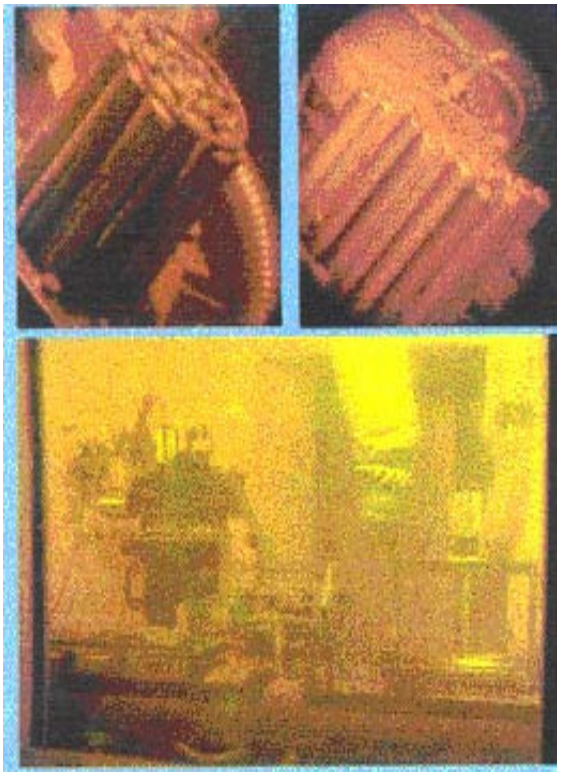
A detailed manual was prepared giving the working principle, calculation of creep measurement process, step-by-step procedures, trouble shooting and information about hardware and software. The manual also mentions about the checklist, which should be filled up for qualifying the operation.

The system was qualified by conducting trials in the laboratory. After designing ADC-528, the cards were fabricated and certified for use. A low pass filter circuit was designed and fabricated in a box (filter box). A test setup simulating the fuelling machine movements using rectilinear potentiometer was developed in laboratory. A two day training programme was held at Engg. Hall No.7 on of December 22 and 23, 2003 to impart training to all the site personnel. Control engineers from nuclear power stations attended the training course. Elaborate theoretical as well as practical training was given. At the end of the programme TMAC (ver 3.0), comprising of ADC-528 card, filter box, cable, software installation disc and user's manual, was handed over to all trainees by Mr G. Govindarajan, Director, A&M and E&I groups, BARC.

Mr R.G. Agrawal, Head, RTD, Mr R.J. Patel, Head, Fuel Handling Design & Development Section, RTD, Mr Vijay Kumar, ACE (Fuel & Fuel Channel), NPC, Mr Rites Ranjon, system designer, RTD, and Mr S. Bhattacharya, RTD, were present during the occasion.

BARC DEVELOPS REMOTELY OPERABLE AUTOMATIC LASER CUTTING SYSTEM

Nuclear fuel bundles and other reactor components need to be cut in Radioactive Hot Cells for post irradiation examination and for reprocessing. The cutting operations are presently being done manually through manipulators by conventional techniques. Therefore, there was an urgent need to develop a laser cutting system for Hot Cell applications.



Pictures show an irradiated PHWR fuel bundle before dismantling (top left), the individual fuel elements after dismantling (top right) and the laser cutting machine inside the hot cell (bottom)

BARC has now developed a special laser cutting system for dismantling of PHWR fuel bundles and commissioned it in the Hot Cells at Post Irradiation Examination Division. The laser cutting process provides the advantage that the laser beam can be taken inside the hot cell through fiber optics and can be manipulated easily.

The laser cutting system receives the fuel bundle on a hoist platform which lifts it up to the level of the gripper assembly platform through a motorized system. The PHWR fuel bundle is then pushed on to the gripper assembly and held by specially designed jaws operated pneumatically. The optical sensor then senses the orientation and location of the bundle and adjusts the CNC zero point accordingly. The laser cutting torch which operates on the CNC platform cuts the tie-plate at one end of the PHWR fuel bundle as per a pre-programmed cutting trajectory. After this, the bundle is rotated automatically by 180^{o} and similar operations are repeated to cut the ether tie-plate and all the fuel elements are released for further processing. The whole process is controlled by a computer and is operated by a single switch. Manual operation of laser cutting is also possible, if required. Computer control makes it possible to handle different bundle geometries by modifying the software. Any individual element can be separated by cutting only the required portion of the tie plate. Components like optical sensor, fiber, switches and cables which go inside the hot cell were tested for irradiation stability before final selection. The modular concept of the system makes its maintenance easy inside the hot cell. More than 25 PHWR bundles were laser cut during pre-commissioning trials to ensure the reliability of the system.

All the PHWR fuel bundles received from power reactors and stored at PIED have been dismantled in two days which otherwise would have taken 3-4 months. The machine at present can cut 2-3 bundles per hour and this can be increased if required. The laser machine along with the fiber optic delivery system was supplied by Centre for Advanced Technology, Indore. The system was developed by a team consisting of Mr Goswami and Mr A Chatterjee of Laser Processing & Advanced Welding Section, Mr S Gangotra, Mr A Bhandekar, J.N. Parab and K.C. Sahoo of Post Irradiation Examination Division, Mr K Jayarajan of Division of Remote

Handling & Robotics, Dr Sailesh Kumar of Laser & Plasma Technology Division, Mr Munish Chandra, Mr K.K Prasad and Mr H.B. Kulkarni of Nuclear Recycle Group and Dr T.P.S Nathan from CAT, Indore. The entire system from feasibility studies to concept to final commissioning was engineered indigenously, and coordinated by Laser Processing & Advanced Welding Section, Nuclear Fuels Group, BARC.

PARALLEL COMPUTING AND ITS APPLICATION IN SCIENCE AND ENGINEERING (PASE)

BARC has been involved in the development of hardware and software for supercomputing applications in Science and Engineering for more than two decades. As a part of this ongoing programme, Director, BARC, identified a few core areas, where in-house efforts are essential for new generation software development. Reactor engineering design, neutronics, radiation transport, computational fluid dynamics (CFD), electromagnetics and materials modelling are some of the key areas of interest to BARC. As part of this endeavour, a DAE meeting on "Parallel Computing and its Application in Science and Engineering (PASE)" was organised jointly by Theoretical Physics Division and Computer Division at BARC during November 27-28, 2003. This meeting was supported by Board for Research in Nuclear Science (BRNS). BARC' s scientists and engineers involved in this activity were invited to present their work at this meeting. Scientist in other DAE units like Institute of Plasma Research (IPR), Institute of Mathematical sciences (IMS), Harish Chandra Research Institute (HRI) and Scientific institutions like Aeronautical Development Agency (ADA), National Centre for Medium Range Weather Forecast (NCMRWF), and Vikram Sarabhai Space Centre (VSSC), engaged in high performance computing were also requested to participate and present their work in this meeting.

Mr B. Bhattacharjee, Director, BARC, inaugurating the meeting on "Parallel Computing and its application" in Science and Engineering (PASE)

At the inaugural function seated on the dais from left to right are : Mr H.K. Kaura, Associate Director, E&I Group, BARC, Mr G. Govindarajan, Director, A&M and E&I Groups, Mr B. Bhattacharjee, Director, BARC, and Mr P.S. Dhekne, Head, Computer Division, BARC

Thirty-eight papers in the fields like reactor related simulations, electromagnetic solvers, CFD, Molecular Dynamics, Monte Carlo, ANUP AM systems, Experience in using parallel system & application in chemistry, etc., were discussed in eleven sessions spread over two days. A special evening lecture entitled "Computers & Needs : Then and Now" by Prof. R. Chidambaram, Principal Scientific Adviser to Govt. of India, was also organised on this ocassion. The meeting was formally inaugurated by Mr B. Bhattacharjee, Director, BARC. The meeting ended with a pannel discussion.

PHOSPHATIC RARE ELEMENT EXTRACTION (PREE) INAUGURATED AT KOCHI

Dr Anil Kakodkar, Chairman, AEC and Secretary, DAE, inaugurating the PREE Test Facility at RED (IREL), Kochi

Dr Anil Kakodkar, Chairman, Atomic Energy Commission and Secretary, Department of Atomic Energy, inaugurated the 'PREE' Test Facility setup by BARC at the Kochi unit of IREL on November 20, 2003. The Phosphatic Rare Element Extraction (PREE) programme forms a thrust area of work at the front end of the fuel cycles. The test facility will be testing individual fertiliser acids for rare element separation using innovative processes developed by BARC. It also has a large circular mixer-settler of proven industrial design made from a corrosion-resistant non-metallic material 'KESTRA'. Graphite heat exchangers have been commissioned for pre-heating process streams. Facilities for pre-treatment by carbon adsorption and post treatment by enhanced gravity separation are integrated into the test rig. A state-of-art instrumentation and control system has been installed. The installation of the facility represents a milestone in industrial development of R&D originating at BARC on rare element recovery from secondary resources.

REGIONAL WORKSHOP ON RADIATION PROCESSING OF NATURAL POLYMERS FOR HEALTH CARE APPLICATIONS

An IAEA/RCA Regional Workshop on "Radiation Processing of Natural Polymers for Health-care Applications" was organised by BARC, during November 3-7, 2003 at Hotel Tulip Star, Juhu, Mumbai. The workshop was organised to bring together the scientists/ technologists working in the area of modification of natural polymers by radiation processing for health care applications

Director, BARC, inaugurating the IAEA/RCA Workshop

to review the ongoing research activities and plan the future strategies for developing commercial applications. 16 scientists from Bangladesh, China, India, IAEA, Indonesia, Japan, Korea, Malaysia, Philippines, Thailand and Vietnam attended the meeting. The inaugural function was held on November 3, 2003. Dr S. Sabharwal, Workshop Coordinator, welcomed the delegates. Dr. V. Venugopal, Associate Director, Radiochemistry & Isotope Group, BARC, in his opening remarks, presented the current status of radiation processing activities being pursued in India using gamma radiation sources and electron beam accelerators. Mr B. Bhattacharjee, Director, BARC, inaugurated the workshop and, in his inaugural address, highlighted the role of radiation technology in modification of polymers for developing industrial applications such as crosslinking of wire and cable, thermo- shrinkable materials, curing of tyres and household pipes. He emphasized that radiation processing of natural polymers is an emerging area as these polymers, due to their unique structure, bio-degradability, biocompatibility and non-toxicity, are now being explored for potential applications in agriculture, food, medicine and cosmetic industries. The important natural polymers that have the potential to be modified include cellulose, starch, chitin-chitosan, alginates, cauageenans and their derivatives, which occur abundantly in the South-East Asia region. Dr A.G. Chmielewski, Radiation Processing expert from IAEA, thanked BARC for the. support being rendered to the RCA activities and for organising this workshop in India. The technical discussions at the workshop included the presentations of individual country report highlighting the achievements in the applications of radiation processed natural polymers in health care applications that have been commercialised, the emerging applications and opportunities in the newly emerging areas. At the workshop, besides the discussions regarding the subject matter, special lectures were also arranged regarding the "Past, Present and Future Applications of Radiation Technology" by Dr A.G. Chmielewski, IAEA, "Radiation Processing of Polymers including Natural Polymers in Japan" by Dr. M. Tamada and "Radiation Technology Activities in India" by Dr Sunil Sabharwal.

Sitting from left to right are: Dr S. Sabharwal, Dr Venugopal, Dr B. Bhattacharjee, Director, BARC, Dr A. Chmielewski, Dr M. Tamada, Mr S.P. Ramani and the participants of the workshop

ONE-YEAR HEALTH STIPENDIARY TRAINING COURSE

Health Physics Division conducts One Year Training Course for graduates in Physics and Chemistry disciplines for imparting training in Health Physics and radiological safety sciences. On successful completion of the training, these trained professionals are posted to the Health Physics Units of different DAE Units including NPCIL. The IX batch of this training programme, consisting of 48 trainees recruited by BARC and NPCIL, graduated in January 2004. The valedictory function for this training batch was held on January 20, 2004 at the auditorium of "Radiation Protection Training and Information Centre, HPD" at CT&CRS building, Anushaktinagar.

Mr A.R. Gore, Senior Executive Director (O), NPCIL delivering the valedictory address

Mr A. R. Gore, Senior Executive Director (Operations), NPCIL, graced the occasion as the chief guest, and Mr H.S. Kushwaha, Director, Health, Safety and Environment Group, BARC, presided over the function. Other dignitaries present included Dr K.S. Parthasarathy, Secretary, AERB, Heads of Divisions of HS&E Group, BARC, senior officers of AERB and NPCIL.

The valedictory function commenced with a traditional welcome by Mr M.L. Joshi, Head, Power Projects Safety Section, HPD, BARC, followed by presentation of bouquets to the dignitaries. In his welcome address, Mr Joshi highlighted the responsibilities of a Health Physics Professional in ensuring safety and stressed on the necessity of a system of reporting the correct information on safety related topics in a scientific spirit so that no compromise is made in implementing safety procedures and inculcating a safety culture. In this context, he reminded the young graduates of the need of their commitment to the application of safety standards and meeting the regulatory requirements.

In his introductory remarks, Mr R.M. Sharma, Head, Health Physics Division, BARC, emphasised on the need of removing the "unfounded fear of excessive risk of radiation" from the minds of the workers through education and training and also to motivate them to handle radioactivity and radiation sources with utmost care. He reminded that the role of a trained Health Physicist is similar to that of a licensed driver – to guide the workers on the safe path to achieve production without compromising on safety. He expressed confidence, quoting his vast experience in nuclear power stations, that following safe work procedures and radiation protection rules, the Health Physicist will be contributing to the plant management to improve performance and to achieve the goal of "total safety". He further emphasised on the need of pursuing R&D activities related to improving safety and updating safety procedures to international standards; incorporating the safety practices prevailing in the latest units elsewhere in the world.

During his presidential address, Mr H.S. Kushwaha, Director, Health, Safety & Environment Group, BARC, briefly touched upon the genesis of the training programme which started in 1989. He expressed satisfaction that the syllabus and course structure of this training

programme are in line with IAEA standards and that a well defined evaluation procedure is adopted to assess the trainees in theory and practicals in addition to their field performance during "On the Job Training". He noted that those who had passed out of the earlier batches are shouldering important responsibilities and doing well in their career advancement. He expressed satisfaction over the excellent performance of the present batch. He also briefly mentioned about the efforts to start the X batch of training in the immediate future at the Radiation Protection Training & Information Centre.

Mr H.S. Kushwaha, Director, Health, Safety & Environment Group, BARC, delivering the presidental address. Seated on the dais (from L to R) Mr R.M. Sharma, Head, Health Physics Division, BARC, Mr A.R. Gore, Senior Executive Director (O), NPCIL, Dr K.S. Parthasarathy, Secretary, AERB, and Mr M.L. Joshi, Head, Power projects Safety Section, HPD, BARC

Mr A.R. Gore, Senior Executive Director (O), NPCIL, delivered the valedictory address. He congratulated all the trainees for their meritorious performance in the training and reminded them of the responsibilities which they have to shoulder in the long span of their career with the organisation. He also stressed on the importance of teamwork especially on the commitment to reduce the ' collective dose' in the Indian Nuclear Power Plants. He reiterated that the role of a good Health Physicist should be proactive to achieve the targets of the units while not compromising on safety procedures. He advised them to interact closely with the plant operators to identify and solve the problems encountered during the operation and maintenance of the plant and contribute their might for the overall improvement in performance.

Dr K.S. Parthasarathy, Secretary, AERB, presented "AERB awards to the merit holders of the batch" which included a certificate of appreciation and cash awards. Mr Mudgal Barunkumar and Mr Vikas R. Ghadigaonkar won the first and second awards respectively. Dr Parthasarathy gave a brief outline of the objectives of AERB including the emphasis on the review of safety related training programmes, encouraging safety research and promoting and funding for safety related activities. He advised the youngsters to browse through the website to keep themselves updated on the developments in the field of nuclear sciences; especially in the fields related to the safety aspects. After the valedictory address, Mr A. R. Gore and Mr H.S. Kushwaha presented ' certificates of successful completion of the training course' to all trainees.

The function concluded with a vote of thanks proposed by Mr K. Narayanan Kutty, Officer-in-Charge, Training Group, HPD. He expressed deep gratitude to the senior officers of the Group for their guidance and keen interest in the activities of the Training Group. He appreciated the cooperation and commitment shown by the course coordinators and faculty members. He noted that the successful completion of such a course was the net result of teamwork and concerted effort of a large number of agencies. At this juncture, he had a special word of appreciation to the assistance received from 18 Health Physics Units of different DAE facilities in arranging the On-the-Job-Training and the wholehearted cooperation on the part of the authorities of these units in extending the necessary training facilities. He made special mention of the fact that some of the trainees, who had their basic education in regional

languages, had fared well in the training. He attributed this to the extra efforts put on by the faculty members as well as the trainees themselves.

MEETING ON POSITRON ANNIHILATION SPECTROSCOPY

A discussion meet on positron annihilation spectroscopy, organised by Radiochemistry Division, was held at BARC on November 7, 2003. There were 57 registered delegates out of which 13 were from other DAE units and Universities. The scientific programme constituted nine invited talks encompassing a wide spectrum of research areas and a panel discussion.

The programme was inaugurated by Dr V .C. Sahni, Director, Physics Group, BARC, and Director, CAT. Dr V .K. Manchanda, Head, Radiochemistry Division, BARC, welcomed the participants and highlighted the need for the discussion meet and the initiatives taken by Radiochemistry Division. Dr V. Venugopal, Associate Director, Radiochemistry and Isotope Group, BARC, delivered the presidential address and pointed out the importance of positron spectroscopy in material sciences, especially its potential role for characterisation of nuclear materials. Prof R. M. Singru delivered the key note address by recollecting the growth of this field in condensed matter physics, material sciences and Positronium Chemistry .He also touched upon the recent advances in this area such as development of slow positron beam and other multiparameter techniques. Dr P.K. Pujari, Convener, proposed the vote of thanks.

The invited talks included a wide spectrum of research areas using positron annihilation spectroscopy. In the area of novel materials such as cuprate superconductors and CMR Mangnites, the utility of positron technique in measuring the local electronic structure and defects was demonstrated. The advent of slow positron beam has widened the scope of positron research. Its unique applicability in studying the surfaces and buried interfaces in thin films was presented. The area of Positronium Chemistry, i.e. the formation probability and its chemical interaction, is quite fascinating. A number of studies pertaining to its behaviour in molecular solids and liquids were presented. Studies on microstructure characterisation of polymers and characterisation of electronic structure of hidden surfaces in catalysts were some of the highlights of the unique applications of positronium atom as a probe in molecular solids. Similarly, work on nanopore characterisation in membranes (produced by ion beam irradiation), study of water diffusion through free-volume measurement in biopolymers (used as contact lens) and structure-property correlations in nanoparticles were presented during the meeting, in addition to the advances in instrumentation and development of new algorithm for positron data analysis.

The group discussion was conducted by Dr B. Vishwanathan with Dr V .K. Manchanda, Prof R.M Singru and Dr G.P. Das as panel members. The highlight of the discussion was identification of challenging areas in material sciences, molecular solids and liquids, membranes and applications of slow positron beam. The need for collaboration between national laboratories and Universities was highlighted. It was also felt that the utility and the strength of this technique in interdisciplinary areas of research should be brought to the notice of researchers working in other disciplines from academic institutions as well as industry. There were suggestions to explore the possibility of bringing out a proceeding of this meeting. Majority of the participants, especially the young research scholars, felt that they have benefited from this meeting and it was suggested that such meeting should be held every year.

भा.प.अ. केंद्र के वैज्ञानिकों को सम्मान / BARC SCIENTISTS HONOURED

• डॉ. धुर्बा ज्योति बिस्वास, लेज़र एवं प्लाज्मा प्रौद्योगिकी प्रभाग, को हाल में ही ट्रियस्ट,इटली में छः साल की अवधि के लिए सीनियर एसोसियेशन ऑफ दि इन्टरनेशनल सेन्टर फॉर थियोरेटिकल फिज़िक्स नामक पुरस्कार से सम्मानित किया गया। इस अवधि के दौरान उन्हें यात्रा एवं अन्य खर्चों के अतिरिक्त ICTP, ट्रियस्ट में तीन शोध-विद्यार्थियों को तीन माह के लिए वैज्ञानिक कार्यक्र म / अनुसंधान में सम्मिलित होने हेतु नामांकित करने का अधिकार भी निहित है।

Dr Dhurba Jyoti Biswas of Laser & Plasma Technology Division, BARC, has recently been awarded the Senior Associateship of the International Centre for Theoretical Physics, Trieste, Italy, for a period of six years. Besides the allocation of fund for his use towards travel and other expenses during this period, this award also entitles him to nominate a maximum of three of his Ph.D. students to participate in the scientific activities/research at ICTP, Trieste, for a period of three months.

• डॉ. पी. वी. वर्दे अनुसंधान रियक्टर सेवाएं प्रभाग को दिसंबर 18-20, 2003 के दौरान इन्डियन नेशनल साइन्स अकादमी,नई दिल्ली में आयोजित ICQRIT-2003 में "एप्लिकेशन ऑफ रिलाइब्लिटी इन्जीनियरिंग टु दि डेवलोपमेंट ऑफ इन्टेलिजेंट ऑपरेटर एडवाइज़री सिस्टमस् फॉर इन्डियन न्यूक्लियर रियक्टस् ", के लिए "पायोनियरिंग रिसर्च इन रिलाइब्लिटी इन्जीनियरिंग" नामक पुरस्कार से सम्मानित किया गया । इस पुरस्कार में, जो कि ICQRIT-2003 के विदाई अवसर पर ICQRIT की प्रबन्धक कमेटी एवं सोसाइटी फॉर रिलाइब्लिटी इन्जीनियरिंग, क्वालिटी एन्ड ऑपरेशन मेनेजमेंट,दोनों ने मिलकर प्रदान किया, प्रशस्ति -पत्र, स्मारिका एवं बधाई निहित है।

डॉ. वर्दे ने वर्ष 1984 में प्रशिक्षण केंद्र के 27वें बैच से स्नातकीकरण किया। इन्होंनें 1995 तक ध्रुवा रियक्टर प्रचालन के लिए शिफ्ट इन्जीनियर के पद पर काम किया। वर्ष 1996 में इन्हें IIT मुंबई के द्वारा पीएचडी (इन्जी) से पुरस्कृत किया गया। रिसर्च रियक्टर ऑपरेशनस् प्रोबेबिलिस्टिक सेफ्टी एसेसमेन्ट, डेवलोपमेंट ऑफ इन्टेलिजेंट ऑपरेटर सर्पोट सिस्टमस् फार रिसर्च रियक्टर एवं पावर प्लांट आदि में इनकी विशेषज्ञता है।

Dr P.V. Varde of Research Reactor Services Division, BARC, was conferred with an award for the "Pioneering Research in Reliability Engineering" for "Application of Reliability Engineering to the Development of Intelligent Operator Advisory Systems for Indian Nuclear Reactors", at the ICQRIT 2003, held at Indian National Science Academy, New Delhi, during December 18-20, 2003. The award, which was jointly given by the organising committee for ICQRIT and the Society for Reliability Engineering, Quality and Operations Management, comprises of a citation, memento and felicitation during the valedictory function of ICQRIT-2003.

Dr Varde has graduated from the 27th batch of Training School in the year 1984. He worked as a shift engineer for Dhruva reactor operations till 1995. In 1996, he was awarded Ph.D. (Engg.) by IIT, Bombay. His specialisations are research reactor operations, probabilisitc safety assessment, development of intelligent operator support systems for reserach reactor and power plants.

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