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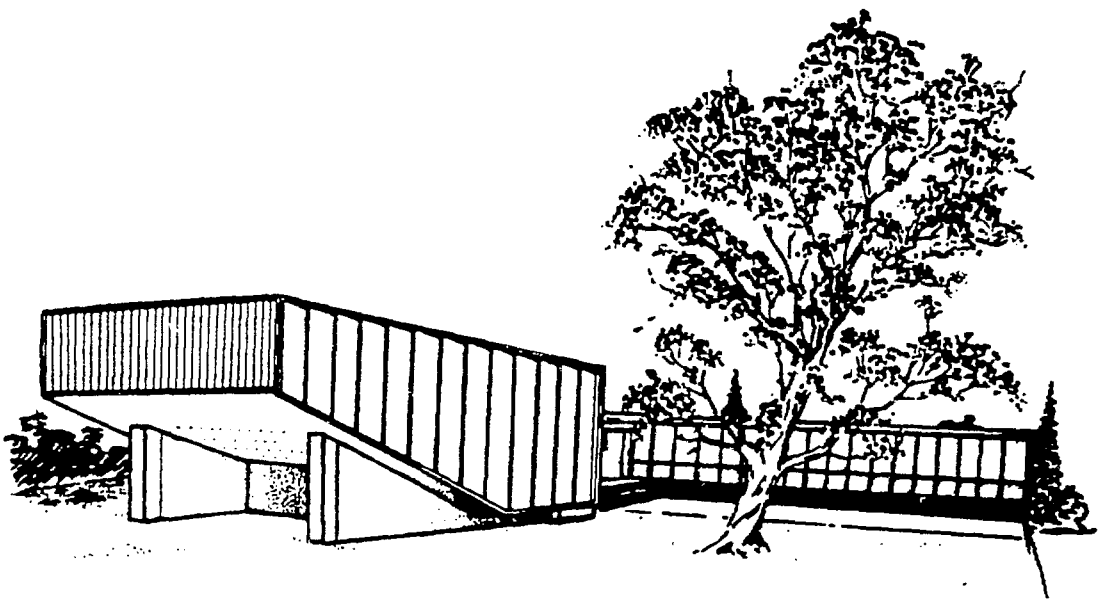
13th AINSE RADIATION BIOLOGY CONFERENCE
2 - 4 OCTOBER, 1991

LUCAS HEIGHTS - AINSE THEATRE



CONFERENCE HANDBOOK

(Program, Abstracts and General Information)

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AUSTRALIAN INSTITUTE OF NUCLEAR SCIENCE AND ENGINEERING

13TH AINSE RADIATION BIOLOGY CONFERENCE 1991

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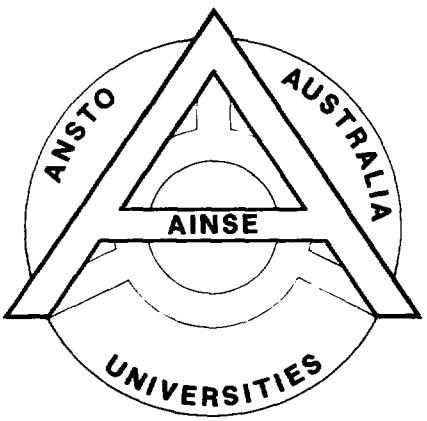

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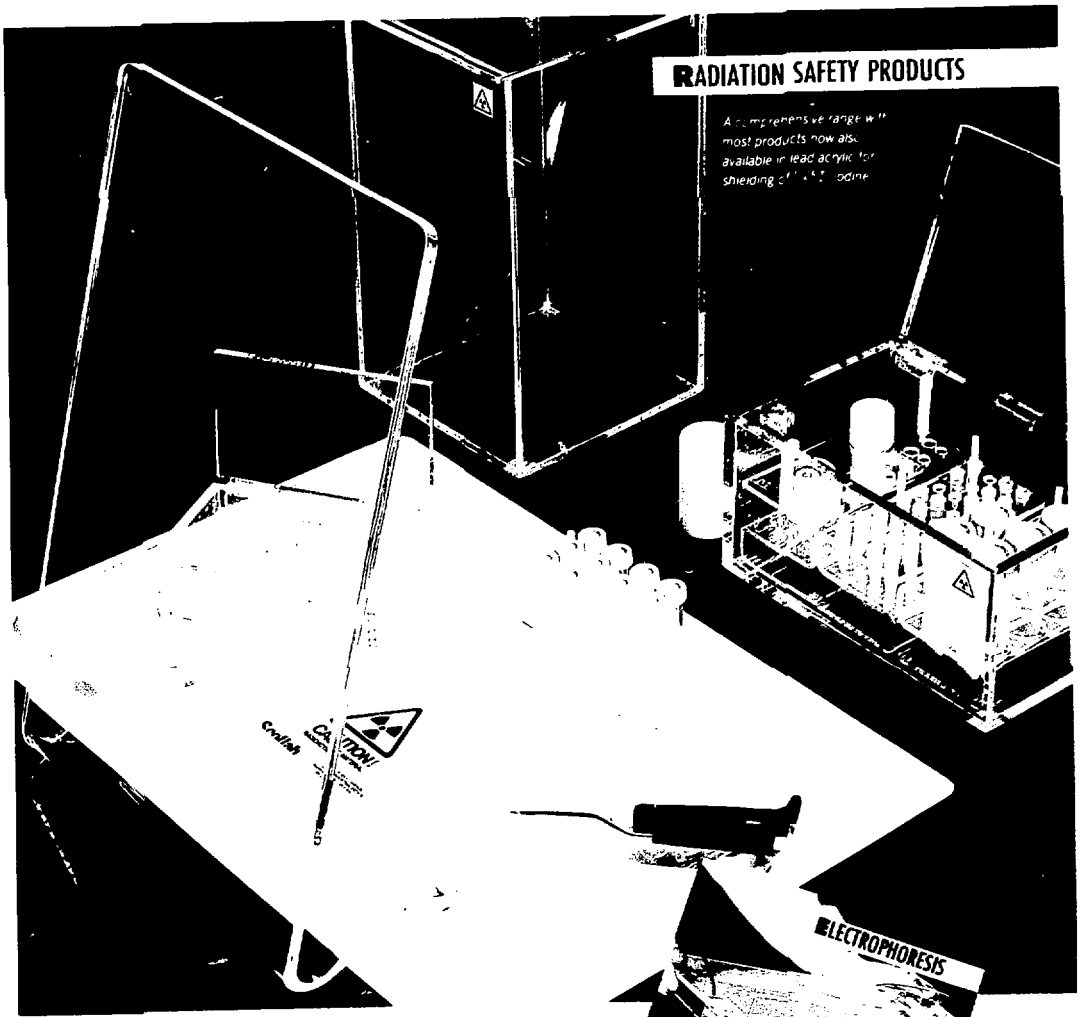

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SUMMARY

Wednesday 2 October 1991

10.30 - 10.40	Opening Remarks
	Conference President: Professor M.F. Lavin (Q.I.M.R.)
Session I	Radiation Induced Lesions
10.40 - 11.40	Chairman: Professor M.F. Lavin (Q.I.M.R.)
11.40 - 12.00	Morning Tea
Session II	Radiation Responses
12.00 - 1.00	Chairman: Dr. M. Fenech (CSIRO)
1.00 - 2.00	Conference Lunch
Session III	Introducing ANSTO
2.00 - 2.30	Chairman: Dr. W.M. Zuk (ANSTO)
2.30 - 2.50	Afternoon Tea
2.50 - 5.15	Tour ANSTO
Session IV	Poster Session
5.15 - 6.30	
6.30	BBQ

Thursday 3 October 1991

Session V	Apoptosis (Programmed Cell Death I)
9.00 - 10.30	Chairman: Dr. I. Radford (Peter MacCallum C.I.)
10.30 - 11.00	Morning Tea
Session VI	Apoptosis (Programmed Cell Death II)
11.00 - 12.20	Chairman: Dr. R. Martin (Peter MacCallum C.I.)
12.20 - 1.00	Lunch
Session VII	Radiation Induced Chromosomal Changes
1.00 - 2.00	Chairman: Dr. S.J. Prosser (ANSTO)
2.00 - 2.15	Break
Session VIII	Symposium - Low Level Radiation Exposure: Dosimetry, Genetics & Radiobiological Consequences I
2.15 - 3.25	Chairman: Professor M.F. Lavin (Q.I.M.R.)
3.25 - 3.45	Afternoon Tea
Session IX	Symposium - Low Level Radiation Exposure: Dosimetry,
3.45 - 5.15	Genetics & Radiobiological Consequences II
	Chairman: Mr. D.R. Davy (ANSTO)
Session X	Poster Session
5.15 - 6.30	
7.00	Conference Dinner

Friday 4 October 1991

Session XI	Clinical Applications of Radiation I
9.00 - 10.30	Chairman: Dr. R. Lambrecht (ANSTO)
10.30 - 10.50	Morning Tea
Session XII	Clinical Applications of Radiation II
10.50 - 12.20	Chairman: Dr. K.J. Brown (ANSTO)
12.20 - 12.30	Closing Remarks
	Conference President: Professor M.F. Lavin
12.30 - 1.00	Lunch
1.00	Depart Lucas Heights via airport
2.00 - 4.15	Tour Medical Cyclotron and Nuclear Medicine,R.P.A.
4.45	Arrive Sydney airport

Wednesday 2 October 1991 - Lucas Heights

TIME	PAPER NO.	
10.30 - 10.40		Opening Remarks - Professor M.F. Lavin (Q.I.M.R.) Conference President
SESSION I		RADIATION INDUCED LESIONS Chairman: Professor M.F. Lavin (Q.I.M.R.)
10.40 - 11.00	1	Radioprotection by DNA Binding Bibenzimidazoles: Mechanistic Studies. L. Denison, R.F. Martin (Peter MacCallum C.I.)
11.00 - 11.20	2	The Interaction of Oxygen with Photoexcited States of Some DNA-Binding Drugs. P. Nell, R. Cooper (Univ. of Melbourne), R. Martin (Peter MacCallum C.I.)
11.20 - 11.40	3	Amino Acid and Protein Peroxidation by Hydroxyl Free Radicals. S. Gebicki, S. Narita, J.M. Gebicki (Macquarie Univ.)
11.40 - 12.00		MORNING TEA
SESSION II		RADIATION RESPONSES Chairman: Dr. M. Fenech (CSIRO)
12.00 - 12.20	4	Characterisation of a Radiation-Activated DNA-Binding Protein (*irap*-1). B. Teale, K.K. Khanna, D. Findik, H. Beamish, M.F. Lavin (Q.I.M.R.)
12.20 - 12.40	5	Purification and Biochemical Characterization of a Novel Binding Protein Activated by Ionizing Radiation. K.K. Khanna, D.F. Findik, B. Teale, M.F. Lavin (Q.I.M.R.)
12.40 - 1.00	6	^{125}I-Decay in Oligodeoxynucleotides. R.F. Martin, G. D'Cunha (Peter MacCallum C.I.)
1.00 - 2.00		CONFERENCE LUNCH - Stevens Hall Lounge

Wednesday 2 October 1991 - Lucas Heights

TIME	PAPER NO.	
SESSION III		Chairman: Dr. W.M. Zuk (ANSTO)
2.00 - 2.10	7	Introducing ANSTO. W.M. Zuk (ANSTO)
2.10 - 2.30	8	Radiation Biology - Regulatory and Educational Aspects. Nuclear Science Experiments in Schools and Universities - A Vanishing Species. G. Lowenthal (U.N.S.W.)
2.30 - 2.50		AFTERNOON TEA
2.50 - 5.15		Tour ANSTO Research Laboratories
SESSION IV		POSTER SESSION ANSTO Technology Park Building
5.15 - 6.30	9	Radiation-Induced Proteins. D. Liaskou, M.F. Lavin (Q.I.M.R.)
	10	Evidence of Different Complementation Groups amongst Human Genetic Disorders Characterized by Radiosensitivity. P. Chen, M.F. Lavin (Q.I.M.R.), C. Kidson (Mahidol Univ., Thailand)
	11	General Characterization of the Genetic Defect in Ataxia Telangiectasia. H. Beamish, M.F. Lavin (Q.I.M.R.)
	12	Local Control of Murine Melanoma Xenografts in Nude Mice by Neutron Capture Therapy. B.J. Allen, S. Corderoy-Buck, H. Meriaty (ANSTO), D.E. Moore (Univ. of Sydney)
	13	An Intra-Pancreatic and Hepatic Nude Mouse Cancer Xenograft Model for Boron Neutron Capture Therapy. D.E. Moore (Univ. of Sydney), J.L. Mallesch, D. Chiaraviglio, B.J. Allen (ANSTO)
	14	Analysis of Copper-Binding Proteins in Mammalian Lymphocytes using the Radionuclide ^{64}Cu. R. Farrell, J. Camakaris (Univ. of Melbourne), D.M. Danks (Royal Children's Hospital, Melbourne)

Wednesday 2 October 1991 - Lucas Heights

TIME	PAPER NO.	
SESSION IV		POSTER SESSION (cont'd)
	15	Measurements of Radon and Other Solid Radioactive Isotopes in Groundwater in the Perth Metropolitan Area. E. Zainuddin, P. Jennings (Murdoch Univ.)
	16	A Novel Method of Isolating Alpha DNA in the Study of DNA-Binding Bibenzimidazoles as Radiomodifiers. J.M. Tursi, R.F. Martin (Peter MacCallum C.I.)
	17	Iodinated Bibenzimidazoles as Radio-Modifiers: Comparison of Four iodoHoechst Analogues. M. Roberts, D.P. Kelly (Univ. of Melbourne), L. Denison, M. Pardee, R.F. Martin (Peter MacCallum C.I.)
	18	BrUdR Sensitisation: Mechanisms of Strand Cleavage by UV-B. G. D'Cunha, D.J. Fitzgerald, V. Murray, R.F. Martin (Peter MacCallum C.I.)
	19	A Human Melanoma Cell Line (WMXRS-1), Hypersensitive to Ionising and Ultraviolet (UV) Radiation and UV-Mimetic Agents. M. McKay, S. Jones, D. McDonald, G. Mann, R. Kefford (Univ. of Sydney)
	20	Radiation Sensitivity in Schizophrenia. P.R. Bates (Univ. of Queensland)
	46	Design of the Access Control and Radiation Monitoring System for the Austin Hospital Positron Emission Tomography Centre. V. Tran (R.M.I.T.), G.F. Egan (Austin Hospital, Melbourne)
6.30		BBQ - Beside ANSTO Pool

Thursday 3 October 1991 - Lucas Heights

TIME	PAPER NO.	
SESSION V		APOPTOSIS (PROGRAMMED CELL DEATH I) Chairman: Dr. I. Radford (Peter MacCallum C.I.)
9.00 - 9.30	21R Review	Cell Death. J.F.R. Kerr (Univ. of Queensland)
9.30 - 9.50	22	Incidence of Radiation, Hyperthermia, and Drug-Induced Cell Death (Apoptosis) in Neonatal Tissues. G.C. Gobé, B.V. Harmon (Univ. of Queensland) D.J. Allan, C. Johnson (Queensland Univ. of Technology)
9.50 - 10.10	23	Kinetics of Radiation-Induced Apoptosis of Rat Spermatogonia. D.J. Allen (QUT), S.A. Roberts (Paterson Inst.,UK)
10.10 - 10.30	24	Anticancer Drugs Induce Cell Death by Apoptosis or Necrosis. L.I. Huschtscha, M.H.N. Tattersall (Univ. of Sydney)
10.30 - 11.00		MORNING TEA
SESSION VI		APOPTOSIS (PROGRAMMED CELL DEATH II) Chairman: Dr. R.F. Martin (Peter MacCallum C.I.)
11.00 - 11.20	25	Apoptosis in Lymphoid Cells. M.F. Lavin, G. Baxter, S.D. Goldstone (Q.I.M.R.)
11.20 - 11.40	26	The Relationship between Mode of Cell Death and Sensitivity to Radiation-Induced DNA Double-Strand Breakage for Mouse Haemopoietic Cell Lines. I.R. Radford (Peter MacCallum C.I.)
11.40 - 12.00	27	ß-Radiation from Tracer Doses of ^{32}P Induces Massive Apoptosis in a Burkitt's Lymphoma Cell Line. T.H. Forster, D.J. Allan, G.C. Gobé, B.V. Harmon, T.P. Walsh, J.F.R. Kerr (Univ. of Queensland and Queensland Univ. of Technology)
12.00 - 12.20	28	Photo-enhancement of the Mutagenicity of 9-Anilinoacridine Derivatives Related to the Antitumour Agent Amsacrine. L.R. Ferguson, Y. Iwamoto, B.C. Baguley (Univ. of Auckland, NZ)
12.20 - 1.00		LUNCH

Thursday 3 October 1991 - Lucas Heights

TIME	PAPER NO.	
SESSION VII		RADIATION INDUCED CHROMOSOMAL CHANGES Chairman: Dr. S.J. Prosser (ANSTO)
1.00 - 1.20	29	The Conversion of Excision-Repairable DNA Lesion to Micronuclei within One Cell Cycle in Human Lymphocytes. M. Fenech, S. Neville (CSIRO)
1.20 - 1.40	30	Relationship between Chromosomal Aberrations at the First and Second Divisions Following X-Irradiation, and their Association with Clonogenic Survival. R.C. Moore (Peter MacCallum C.I.)
1.40 - 2.00	31	Micronucleus Frequencies in Spleen and Peripheral Blood Lymphocytes of the Mouse Following Acute Whole Body Irradiation. M.F. Fenech (CSIRO), V. Dunaiski, B.J.S. Sanderson, A.A. Morley (Flinders Univ.)
2.00 - 2.15		BREAK
SESSION VIII		SYMPOSIUM: Low Level Radiation Exposure: Dosimetry, Genetics & Radiobiological Consequences I Chairman: Professor M.F. Lavin (Q.I.M.R)
2.15 - 2.45	32R Review	Delayed Effects of Low Level Radiation. K. Lokan (Australian Radiation Laboratory)
2.45 - 3.05	33R Review	Some Aspects of the Background and Radiobiological Consequences of the Chernobyl Acident. R.F. Martin (Peter MacCallum C.I.)
3.05 - 3.25	34R Review	Chernobyl - Evaluation of the Consequences. M.F. Lavin (Q.I.M.R.)
3.25 - 3.45		AFTERNOON TEA

Thursday 3 October 1991 - Lucas Heights

TIME	PAPER NO.	
SESSION IX		SYMPOSIUM: Low Level Radiation Exposure: Dosimetry, Genetics & Radiobiological Consequences II Chairman: Mr. D.R. Davy (ANSTO)
3.45 - 4.05	35R Review	Biological Dosimetry after Radiation Accidents. J.S. Prosser (ANSTO)
4.05 - 4.25	36R Review	Mutagenic and Carcinogenic Effects of Low Levels of Ionizing Radiation. D.G. MacPhee (La Trobe University)
4.25 - 4.45	37R Review	Radiation Hormesis. K. Brown
4.45 - 5.15		Symposium Discussion
SESSION X 5.15 - 6.30		POSTER SESSION ANSTO Technology Park Building Posters as for Session V
6.30 for 7.00		Depart for CONFERENCE DINNER Sutherland District Trades Union Club, Gymea

Friday 4 October 1991 - Lucas Heights

TIME	PAPER NO.	
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9.00 - 9.30	38R Review	Clinical Applications of Radiation. J.H. Kearsley (Queensland Radium Inst.)
9.30 - 9.50	39	Does Boron Neutron Capture Therapy Regress Cancer? J.L. Mallesch, B.J. Allen (ANSTO), D.E. Moore (Univ. of Sydney)
9.50 - 10.10	40	Neutron Sources for Boron Neutron Capture Therapy. B.V. Harrington, G. Storr, B.J. Allen, R. Godfrey, H. Meriaty (ANSTO)
10.10 - 10.30	41	The Role of Heavy Water in Dose-Depth Enhancement in Thermal Neutron Capture Therapy. N. Blagojevic, B.J. Allen, G. Storr (ANSTO), H. Hatanaka (Teikyo Univ., Japan)
10.30 - 10.50		MORNING TEA
SESSION XII		CLINICAL APPLICATIONS OF RADIATION II Chairman: Dr. K.J. Brown
10.50 - 11.10	42	The Effects of Three Bioreductive Drugs on Cell Lines Selected for their Sensitivity to Mitomycin C or Ionising Radiation. A. Keohane (Peter MacCallum C.I.), J. Godden, I. Stratford, G. Adams (MRC Radiobiology Unit, UK)
11.10 - 11.30	43	Histological Examination of Melanoma Xenografts in the Nude Mouse Model: Pre and Post Neutron Capture Therapy. K. Crotty, D.E. Moore (Univ. of Sydney), J. Mallesch, B.J. Allen (ANSTO)
11.30 - 11.50	44	The ANSTO Body Protein Monitor. N. Blagojevic, B.J. Allen (ANSTO)
11.50 - 12.20	45R Review	Radiopharmaceuticals. R.M. Lambrecht (ANSTO)
12.20 - 12.30		Closing Remarks - Professor M.F. Lavin (Q.I.M.R.) Conference President
12.30 - 1.00		LUNCH

Friday 4 October 1991 - Lucas Heights

TIME	
1.00	Depart Lucas Heights via Airport
2.00 - 4.15	Tour Medical Cyclotron and Nuclear Medicine Royal Prince Alfred Hospital
4.45	Arrive Sydney Airport

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A B S T R A C T S

RADIOPROTECTION BY DNA BINDING BIBENZIMIDAZOLES: MECHANISTIC STUDIES

by

L. Denison and R.F. Martin

Molecular Sciences Group
Peter MacCallum Cancer Institute
481 Lt. Lonsdale Street Melbourne, 3000.
Australia

Abstract

An iodinated bibenzimidazole, iodoHoechst 33258, was previously reported [1] to markedly sensitise DNA and cells to UV-A, exemplifying the potential of iodinated DNA ligands as radiosensitisers; a rational extension of sensitisation by halogenated pyrimidines [2]. However, unlike the latter sensitisers, iodoHoechst 33258 is not a sensitiser of ionising radiation, presumably due to the innate radioprotective properties of the uniodinated ligand. Experiments with purified DNA show that both Hoechst 33258 and Hoechst 33342 decrease the yield the radiation-induced DNA strand breakage. The ligands bind at discrete sites in the minor groove of DNA, and analysis on DNA sequencing gels show pronounced protection at the ligand binding sites, as well as more generalised protection [3]. The extent of protection of strand breakage on plasmid DNA, and the fact that it persists in the presence of 0.5M NaCl (which prevents low affinity ionic binding between the high affinity sites), suggests that the protective effects of bound ligand are not confined to the high affinity binding sites in the minor groove. The mechanism of this generalised protection is unknown, but there is some evidence indicating that the H-atom donation from the ligand may account for the site-specific protection. The extent of protection is much diminished, but still evident, in the presence of 100mM mannitol, a known hydroxyl radical scavenger, indicating that some of the protective effects might relate to DNA damage mediated by direct action. Further evaluation of the mechanisms of protection should enable development of more active radioprotectors, and by elimination of the radioprotective features from halogenated DNA ligands, more effective radiosensitisers.

References

[1] Martin, R.F., Murray, V., D'Cunha, G., Pardee, M., Kampouris, E., Haigh, A., Kelly, D.P., Hodgson, G.S., Radiation sensitisation by an iodine-labelled DNA ligand. Int. J. Radiat. Biol. (1990) 57, 939-946;.

[2] Hutchinson, F., Kohnlein, W., The photochemistry of 5-bromouracil and 5-iodouracil in DNA. Progr. Mol. Subcell. Biol. (1980) 7, 1-42;.

[3] Denison, L., Haigh, A., D'Cunha, G., Martin, R.F., DNA ligands as radioprotectors: Molecular studies with Hoechst 33342 and Hoechst 33258. In press, Int. J. Radiat. Biol. (1991).

THE INTERACTION OF OXYGEN WITH PHOTOEXCITED STATES OF SOME DNA-BINDING DRUGS

by
*#Petronella Nel, *Ronald Cooper and #Roger Martin

*Department of Chemistry,
The University of Melbourne,
Parkville, Victoria.

#Molecular Sciences Group,
Peter MacCallum Cancer Institute,
Melbourne, Victoria.

A series of compounds, based on bisbenzimidazole, was synthesised by the Hoechst Pharmaceutical Company in the early 'seventies. The fluorescence of these drugs was strongly enhanced upon binding to the minor groove of DNA. This has resulted in the widespread use of Hoechst 33258 and Hoechst 33342 (both commercially available) as dyes for the visualisation of both isolated and cellular DNA in biochemistry and cell biology. Further application of these compounds, as potential radiomodifiers, is being pursued in collaborative research between the Peter MacCallum Cancer Institute and The University of Melbourne.

It is well established that Hoechst 33258 and UV light sensitise cleavage of halogenated DNA strands. Inspired by this knowledge, work began with the synthesis of iodo-Hoechst 33258, as a potential radiosensitizer. Iodo-Hoechst 33258 was found to be a potent sensitizer of UVA-induced DNA strand breakage, presumably mediated by the formation of a carbon-centred radical on the ligand upon dissociation of iodine.

Subsequent experiments show that the uniodinated compounds are protectors against radiation-induced DNA strand breaks. These observations have led to the synthesis of ten analogues in an attempt to optimise the design of these radioprotectors (non-halogenated ligands) and radiosensitizers (halogenated ligands).

We wish to determine the factors that are critical for these compounds to be good sensitizers or protectors. Therefore investigations were conducted into the spectroscopic and photodynamic properties of these compounds. From previous work conducted at The University of Melbourne it was observed that solvent, pH and DNA binding affected the fluorescence quantum yields, fluorescence lifetimes, absorption spectra and emission spectra of these drugs.

We then investigated how these drugs interacted with oxygen. It was found that the excited Hoechst molecule acted via an electron transfer mechanism with oxygen to give superoxide. Measurements of photosensitised superoxide production for various Hoechst derivatives, indicated a possible correlation between electron withdrawing substituents and sensitisation of DNA strand breakage by ionising radiation.

Superoxide production was also found to correspond to one specific absorption band of Hoechst 33258 and Hoechst 33342. The number of strand breaks produced by these drugs when excited by various discrete wavelengths of UV light in plasmid pBR322 DNA was measured using agarose gel electrophoresis and laser densitometry. From these results, we determined the role played by electron transfer in the sensitisation or protection of DNA strand breakage.

AMINO ACID AND PROTEIN PEROXIDATION BY HYDROXYL FREE RADICALS

by

S. Gebicki, S. Narita and J.M. Gebicki

Macquarie University
School of Biological Sciences
Sydney, NSW 2109

Abstract

Tissues undergoing oxidant stress accompanied by release of free radicals are protected from damage initially by antioxidant molecules which are sacrificially oxidized. A necessary property of antioxidants is low or zero reactivity of the products of their reactions with free radicals. Until now, proteins were generally believed to be important antioxidants because of their abundance in living organisms, reactivity with oxidizing species and ready replacement. As there was no information on the reactivity of the products of protein - radical reactions, they were generally assumed to be inert.

We have found recently that at least two proteins - bovine serum albumin and lysozyme - acquire reactive hydroperoxide groups after exposure to hydroxyl free radicals. The peroxides are a major product of the interaction, with 40% of $HO^{\cdot}$ radicals generated becoming converted to -OOH groups in BSA and 30% in lysozyme. The peroxides are relatively stable, with a half life in solution of about 1.5 days at room temperature.

Exposure of all the common amino acids to $HO^{\cdot}$ radicals generated by gamma radiation showed that 6 of them - lys, leu, ile, glu, pro and val - also became peroxidized. For these, the peroxide yields were similar to those found in the proteins.

An important property of the protein peroxides is their ability to oxidize a range of compounds. Of these, the most biologically significant are GSH and ascorbate. Both these constitute part of the antioxidant machinery of cells and tissues, necessary for the maintenance of sensitive groups in the reduced state and required in repair of oxidant damage. The finding that proteins exposed to free radicals can oxidize other cell constituents may uncover a new pathway for biological damage initiated by radiation and other oxidants.

Characterisation of a Radiation-Activated DNA-Binding Protein (*irap*-1)

Brett Teale, Kum Kum Khanna, Duygu Findik, Heather Beamish, and Martin F. Lavin.

(Queensland Institute of Medical Research, The Bancroft Centre, Royal Brisbane Hospital Post Office, Herston, Australia.)

The induction of a cellular response by different stress inducing agents, the mechanism through which it occurs, and its relationship to the regulation of genes in normal cellular processes have been of interest to researchers for many years. Study of the initiation, upregulation or inhibition of specific genes in response to cellular stress helps us to understand the complex pathways through which a cell may control its intracellular environment and maintain, or regain, its normal functional processes.

This study is an extension of the research carried out by Singh and Lavin (1990) into an ionising-radiation activated DNA-binding protein. This protein was initially isolated from A-T homozygote cell lines, and was later found to be present in the cytoplasm of untreated control cells and translocated to the nucleus when treated with ionising radiation. It was this constitutively present, cytoplasmic form of the DNA-binding protein that was the basis of this study.

Using the Simian Virus 40 promoter/enhancer region as the ligand for Electrophoretic Mobility Shift Assays, the kinetics of the dose course of the time-dependant localisation were determined. Several stress inducing agents including heat, ultraviolet light, fast neutrons and streptonigrin were substituted for the ionising radiation treatment to elucidate the specificity of the activation response. The response, by translocation of the protein from the cytoplasm to the nucleus, appeared to be specific only to those agents that produced oxygen free radicals, viz ionising radiation, fast neutrons, and the radiomimetic agent, streptonigrin.

The aim of this study was to develop a purification procedure for the binding factor. The cytoplasmic fraction, extracted from control lymphoblastoid cell lines, was initially subfractionated on the basis of size by chromatography, and then selectively precipitated by ammonium sulphate. The active protein was then further fractionated by the use of Heparin-agarose which binds a variety of active proteins and enzymes with varying affinities. The active elute from the Heparin-agarose column was then dialysed and applied to a DNA-affinity column. The pure protein was then used for a variety of characterisation experiments.

The size of the protein was confirmed, by SDS-Page, to be approximately 47kD. The iso-electric point was determined by iso-electric focussing and shown to be pI 8.0 , indicating a slightly basic structure. Through heat denaturation and renaturation, it was shown that the DNA-binding activity of the protein was heat labile. The major characterisation achieved during this study was the localisation of the protein-DNA contact points through the use of DNase 1 protection mapping.

Reference:

1. Singh SP. and M F. Lavin.'DNA-binding Protein Activated by Gamma Radiation in Human Cells' *Mol. Cell. Biol.* **10**, 5279-5285 (October 1990).

PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A NOVEL BINDING PROTEIN ACTIVATED BY IONIZING RADIATION

by

K.K. Khanna, D.F. Findik, B. Teale and M.F. Lavin

Queensland Cancer Fund Research Unit,
Queensland Institute of Medical Research,
The Bancroft Centre, P.O. Royal Brisbane Hospital,
Brisbane, Australia 4029

Abstract

A recent report from this laboratory described a novel DNA binding protein recognising the specific sequence TGTCAGTTAGGGT which was activated by
ACAGTCAATCCCA
gamma radiation in human lymphoblastoid cells (1). Here we describe the purification of this protein/factor from human placenta.

The purification scheme consists of chromatography of soluble whole cell extract on DEAE Sepharose CL-6B, heparin agarose, and three successive DNA affinity columns. The DNA affinity matrix was prepared by coupling ligated complementary synthetic oligonucleotides containing the recognition sequence to CNBr activated sepharose CL-4B (2). The DNA binding activity was monitored throughout the purification by gel mobility shift assay using end-labelled synthetic oligodeoxynucleotides. The purified preparation consists of three major polypeptides with molecular weights of 70 kDa, 47 kDa, 31 kDa bound specifically to the recognition sequence as determined by UV crosslinking and south western blotting. The sequence of these proteins will be determined and subsequently the cDNA clones will be isolated. This will help us to delineate the relationship between these DNA binding proteins, which might advance our knowledge on regulation of gene expression in ataxia telangiectasia.

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^{125}I-DECAY IN OLIGODEOXYNUCLEOTIDES

by

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Abstract

The availability of synthetic oligodeoxy nucleotides provides the opportunity to study the effects of ^{125}I in DNA with added precision and detail, compared to an earlier study [1]. We have designed a template/primer system which enables incorporation of ^{125}I-dC into a defined location in a 31mer:-

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5' 32P *CCTCTTCAAGAATTCTCACT          3'
3'      GGAGAAGTTCTTAAGAGTGAGCCGAGGCCAT 5'
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The ^{125}IdCTP is introduced using T4 DNA polymerase and then "chasing" with excess dNTPs (specifically, a 1000 x excess of dCTP).

The main advantage of this approach is that relatively large amounts (a few microcurie) of DNA with both ^{32}P and ^{125}I can be produced quite easily. Consequently, when ^{125}I-induced DNA damage is analysed on DNA sequencing gels, the distribution of ^{32}P-labelled cleavage products can be determined more accurately than in the previous study, which relied on densitometer scans. In particular, the effects of accumulating decay events under various conditions (e.g. temperature, buffer components, free radical scavengers) can be studied more quantitatively. Preliminary experiments have indicated that the yield of strand breaks in the region of ^{125}I-decay is of the order of one break per ^{125}I decay event.

The question of energy migration is also being investigated in the oligodeoxynucleotide system, by studying the effects of inclusion of BrdU nucleotides at various distances from the ^{125}I-dC. Preliminary results indicate that such energy transfer does not extend beyond 10bp, on contrast to an earlier claim [2] of extensive energy migration.

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INTRODUCING ANSTO

by

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RADIATION BIOLOGY - REGULATORY AND EDUCATIONAL ASPECTS

NUCLEAR SCIENCE EXPERIMENTS IN SCHOOLS AND UNIVERSITIES - A VANISHING SPECIES

by

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Abstract

On the professional level, radiation biology and other nuclear radiation applications have held their own. The situation is different with experimental work in educational institutions.

In the senior grades of secondary schools, the Code of Practice for School Experiments issued by the National Health and Medical Research Council tends to discourage experimental work with radionuclides rather than encourage it.[1] School experiments with open sources have vanished from the syllabus, so denying pupils in biology and chemistry the opportunity for meaningful experiments. This is so notwithstanding the important role played by radionuclides in health care.

The large number of tertiary institutions and the considerable diversities in their programmes makes the role of nuclear science experiments in these institutions difficult to assess, however, a definite trend exists. Subjects like radiobiology and radiochemistry are only taught in limited numbers of these institutions, but experimental work in all nuclear radiation applications, not least nuclear physics experiments, tends to rely on outdated equipment and there is a grave lack of personnel with sound, hands-on experience in radionuclide metrology. Efforts to improve this situation are frustrated by shortage of funds and misunderstandings - deliberate or from ignorance - of possible deleterious effects on health from low levels of ionising radiations.[2,3]

It is in the interest of radiation biologists to encourage a substantial strengthening of experimental work with radioactivity in educational institutions. This would have to be done as regards both quality and quantity, and not only in biology, but equally in all other sciences involving nuclear radiations.

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Radiation-Induced Proteins

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Exposure of mammalian cells to stress-inducing agents such as ionizing radiation results in DNA damage, genetic change and cell death. A feature of the stress response of cells is the induction of specific genes or the modification of pre-existing proteins. This induction may be a protective response and includes proteins involved in processes such as DNA repair. However, little is known about specific gene expression or the induction of cellular processing events as a consequence of ionizing radiation exposure. Cells from patients with the human genetic disorder, Ataxia-telangiectasia (AT) may be particularly useful in the study of radiation-induced proteins as they are hypersensitive to the effects of ionizing radiation.

mRNA isolated from gamma-irradiated and non-irradiated control and AT cells was translated *in vitro* using a rabbit reticulocyte translation system. Two dimensional polyacrylamide gel electrophoresis was used to analyse the resulting protein products. Radiation-induced proteins were evident.

EVIDENCE OF DIFFERENT COMPLEMENTATION GROUPS AMONGST HUMAN GENETIC DISORDERS CHARACTERIZED BY RADIOSENSITIVITY

by

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Abstract

Radiosensitivity has been demonstrated in ataxia-telangiectasia (A-T), Alzheimer's disease (AD) and Down's syndrome (DS) by examining radiation-induced chromosome aberrations in lymphoblastoid cell lines (1). Cell fusion was employed to investigate the presence of different complementation groups for the radiosensitive phenotype in A-T, AD and DS using frequency of radiation-induced chromosome aberrations as a means of distinguishing different groups. Several complementation groups were found among A-T and AD cell lines (2,3). In the present study, we have investigated the radiosensitive phenotype in cross fusions between A-T, AD and DS and established that at least 8 complementation groups exist among 11 cell lines tested. These findings suggest that this group of radiosensitive syndromes might have some common genetic defect.

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GENERAL CHARACTERIZATION OF THE GENETIC DEFECT IN ATAXIA TELANGIECTASIA

by

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Abstract

Ataxia telangiectasia (A-T) is an autosomal recessive human genetic disorder characterised by progressive cerebellar ataxia, oculocutaneous telangiectasis, immune deficiency and increased sensitivity to ionizing radiation. The exact nature of this disorder is unknown.

Two approaches have been used to gain an understanding of the biochemical defect in A-T.

a: In order to clone a gene (or genes) which maybe altered in AT patients, subtractive hybridisation was utilized. An aqueous biotin-streptavidin procedure including a PCR step to produce the subtracter DNA; has led to the isolation of a number of possible candidates.

b: A-T has been shown to have radioresistant DNA synthesis as well as a profound G_2 block post-irradiation, as determined by flow cytometery. Both of these characteristics point to the fact that the cell cycle control mechanisms within A-T cell lines as compared to control cell lines may be altered. At the G_1/S and G_2/M interface of the cell cycle, there are a large number of control factors involved. The principal one being cyclin, which has been shown to play a role at both of these restriction points. Also the maturation promotion factor has a crucial controlling effect over the entry of a cell into mitosis, cyclin B and cdc 2 are the main protein factors involved; also cdc 25 which may be the tyrosine phosphatase which controls the phosphorylation state of maturation promotion factor (MPF) and allows entry into mitosis. The levels of expression of these control elements have been studied.

LOCAL CONTROL OF MURINE MELANOMA XENOGRAFTS IN NUDE MICE BY NEUTRON CAPTURE THERAPY

by

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Abstract

The systemic administration of enriched ^{10}B-BPA results in selective uptake by melanoma xenografts in the nude mouse model. The boron-10, located in the tumour cells, acts as a radiation sensitiser for thermal neutron irradiation. The effect of ^{10}BPA as a neutron sensitiser is clearly observed for the first time in the nude mouse model for a tumour cure endpoint when an incident neutron fluence of 1.1×10^{13} n cm^{-2} is combined with an average of 30 ppm enriched boron-10 concentration in the tumour. A therapeutic gain of 6 is achieved.

The NCT treated mice have been observed for more than 200 days. Tumour recurrence was observed at 30 and 200 days. There is no evidence in our experiments to suggests that tumour cures can be ascribed to the neutrons alone.

As a consequence, we have demonstrated that the nude mouse model is a viable model for testing the efficacy of neutron capture therapy. In the case of the Harding-Passey melanoma xenograft model, we have shown that a single NCT treatment can bring about complete local control, without inducing significant skin damage.

Complete local control is a manifestation of the high LET radiation released after neutron capture by boron-10, and does not result from a equal fluence of neutrons alone.

Acknowledgments

Work supported in part by the Government Employees Assistance to Medical Research Fund.

AN INTRA-PANCREATIC AND HEPATIC NUDE MOUSE CANCER XENOGRAFT MODEL FOR BORON NEUTRON CAPTURE THERAPY

by

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Abstract

The cure of many cancers still depends on early detection and surgical excision. The present protocols for the treatment of hepatic and pancreatic cancers have poor selectivity and this compromises their effectiveness. Our aim has been to establish and then utilise a new experimental model in order to assess the potential of Neutron Capture Therapy for the management of pancreatic and liver carcinomas.

Subcutaneous inoculation of pancreatic and hepatic tumour cell lines into the flank of nude mice has many limitations. Inoculation of cancer cells into their organ of origin may produce a different, but potentially more realistic, behaviour of that tumour type. This may then provide a superior indication of the potential of targeting these cancers with boron compounds.

Harding-Passey murine melanoma cells were injected between the peritoneal membranes containing the pancreas, or directly into the parenchyma of the right lobe of the liver, in nude mice. One week after inoculation, 12mg of BPA-fructose was injected intraperitoneally, then the mice were sacrificed at intervals up to 24 hours.

The primary tumour obtained from the inoculation site is a discrete nodule which can be easily separated from the surrounding healthy tissue, providing enough material for boron analysis by ICP-AES.

The pancreatic inoculations are very successful and the tumours possess faster growth kinetics than subcutaneously injected control tumours. The pancreas has a greater uptake of BPA-fructose than the tumour, for both models. The hepatic inoculations are less successful and produce smaller tumours but the tumour/liver boron ratio is in the range of 2-4. Because the tumour/pancreas ratio is less than 1 for intrapancreatic melanoma, hormonal stimulation/ blocking and/or inhibition techniques will be required to reverse this situation.

Acknowledgments

This work was supported in part by the Government Employees Assistance to Medical Research Fund.

ANALYSIS OF COPPER-BINDING PROTEINS IN MAMMALIAN LYMPHOCYTES USING THE RADIONUCLIDE, ^{64}CU

by

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Abstract

The aim of the studies is to determine the normal intracellular copper transport pathways in mammalian lymphocytes, and to elucidate the defects in cells carrying mutations which disturb copper transport (e.g. Menkes' disease). Cultured lymphocytes are incubated in growth medium containing the radionuclide, ^{64}Cu (from ANSTO), and cell extracts prepared. The development of a FPLC gel permeation system has provided a high resolution method for analysing ^{64}Cu-labelled proteins in the cell extracts. It has been found that, in the presence of reducing thiols, ^{64}Cu redistributes to the high affinity heavy metal binding protein, metallothionein. This results in artifactually high levels of ^{64}Cu appearing to be associated with this protein. The use of an anaerobic FPLC system has minimised the observed *in vitro* redistribution.

Using modified FPLC procedures and ^{64}Cu we have carried out "pulse-chase" studies, and identified putative copper transport proteins.

MEASUREMENTS OF RADON AND OTHER SOLID RADIOACTIVE ISOTOPES IN GROUNDWATER IN THE PERTH METROPOLITAN AREA

by

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Abstract

Measurements of radon and other solid radioactive isotopes from 34 bores and wells in the Perth Metropolitan Area give concentrations in the range 483 to 182500 Bq m^{-3} and 101 to 1667 Bq m^{-3}, respectively, with calculated radium concentrations of 17 to 370 Bq m^{-3}.

The method used is a modified version of the rapid measurement technique for Rn(222) concentration in water with a commercial liquid scintillation counter developed by Prichard et al (1977). Every sample from the field is separated into two parts in the laboratory, one is outgassed for 1 hour and the other is processed immediately and counted in the liquid scintillation counter (MINAXI TRI-CARB 4000 series model 4430 and 4450 made by United Technologies Packard Ltd.). The total count due to this sample is assumed to be the count from radon, radium and radon decay products, while the outgassed sample is assumed to give the count due to solid isotopes. The procedure is calibrated using standard solutions of thorium and potassium salts. It provides an inexpensive and reliable method of measuring radon and other solid isotopes in water. A correction is applied to account for the effects of K(40).

The results show that there are elevated radioactivity levels in groundwater from the Perth Hills area.

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A NOVEL METHOD OF ISOLATING ALPHA DNA IN THE STUDY OF DNA-BINDING BIBENZIMIDAZOLES AS RADIOMODIFERS

by

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Abstract

Hoechst 33258 and Hoechst 33342 bind in the minor groove of DNA, at sites comprising 3 or more consecutive AT base pairs[1]. An iodinated bibenzimidazole, iodoHoechst 33258, was previously reported to markedly sensitise DNA and cells to UV-A[2]. However, iodoHoechst is not a sensitiser of ionising radiation. Presumably due to the innate radioprotective properties of the uniodinated ligand.

In an attempt to optimize the design of radiosensitizers (halogenated bibenzimidazoles) and radioprotectors (non-halogenated bibenzimidazoles) we are evaluating a number of newly synthesised Hoechst analogues, by molecular studies with purified DNA and by cell survival assays with cultured cells. The aim of this project is to "bridge the gap" between these two types of experiments by applying DNA sequencing techniques, to evaluate DNA strand breakage induced in intact cells by radiation, with and without added radiomodifiers.

Alpha DNA (344 bp) is a repetitive sequence of approximately 100,000 copies in the diploid human genome. This DNA has been extensively studied and the consensus sequence determined [3]. The sequence homogeneity of alpha DNA enables application of sequencing gel analysis to investigate sequence specificity of damage to DNA in intact cells after exposure to bibenzimidazoles and radiation.

As an extension of an earlier approach [4] we have synthesized an oligonucleotide designed to exploit the affinity chromatography techniques developed for the purification of poly(A) mRNA. The oligonucleotide is comprised of 21 nucleotides complementary to alpha DNA and an oligo$(A)_{20}$ tail. The hybridization of the oligomer and alpha DNA is monitored by native polyacrylamide gel electrophoresis. Unsuccessful attempts have been made to capture the hybrids on affinity chromatography support (cellulose column or magnetic beads) containing oligo dT. This may be due to the oligo $(A)_{20}$ tail annealing to T bases upstream from the hybridized region making it unavailable for capture by the oligo dT chromatography support. We are currently investigating another oligonucleotide, complimentary to part of the 49bp HaeIII/EcoRI restriction fragment of alpha DNA, which we hope will overcome this problem.

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IODINATED BIBENZIMIDAZOLES AS RADIOMODIFIERS: COMPARISON OF FOUR iodoHOECHST ANALOGUES

by

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Abstract

Our earlier studies have shown that iodoHoechst (R_2 = I, R_3 = OH) sensitises DNA to UVA-induced strand breakage [1]. DNA sequencing gels studies showed that the sites of strand breakage coincide with the known ligand binding sites. Marked sensitisation of UVA-induced cell kill was also observed after exposure to iodoHoechst.

We have now prepared three further analogues of iodoHoechst in order to ascertain the effect of the position of iodine substitution in the phenyl ring of the ligand on the extent of sensitisation . The analogues are:-

*ortho*iodoHoechst (R_1=I)
*meta*iodoHoechst (R_2=I)
*para*iodoHoechst (R_3=I)

The three analogues have been compared by assay of UVA-induced single-stranded breaks in pBR322 DNA in the presence of the ligand. The order of decreasing extent of sensitisation is *ortho* > *meta* > *para* > iodoHoechst. Under comparable conditions, the UVA-fluence required to induce the same level of single-stranded breakage varied by a factor of 40 between *ortho*iodoHoechst and iodoHoechst.

Preliminary studies using DNA sequencing gel analysis of strand-breakage, indicates differences in the sites of cleavage between the analogues.

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BrUdR SENSITISATION: MECHANISMS OF STRAND CLEAVAGE BY UV-B

by

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Abstract

Earlier studies have indicated that the sensitisation of BrU-or IU-substituted DNA to strand cleavage by UV-B is mediated by the uracilyl radical, formed by photodissociation of the carbon-halogen bond[1]. It has been proposed that the carbon-centred uracilyl radical is so located in the major groove that it abstracts a H-atom from the 2'-deoxyribosyl carbon of the nucleotide immediately 5' to the halogenated nucleotide. We have used DNA sequencing gels to analyse UV-B induced cleavage in oligonucleotides with Br-U incorporated at specific locations. The results have confirmed that a major component of strand breaks conforms to the above hypothesis. However, the extent and nature of strand cleavage is dependent on the sequence environment[2], and there is evidence for other mechanisms. For example, piperidine treatment of irradiated DNA revealed lesions in nucleotides 3' to the substitution site.

We have also investigated sensitisation by Hoechst 33258 of UV-A-induced cleavage of brominated DNA, in an oligonucleotide which has one ligand binding site and halogenated uracils at various locations. Sensitisation of cleavage is particularly evident at a substitution site 2 nucleotides away from the ligand binding site. Similar studies with other oligomers are being undertaken to determine the extent of energy transfer.

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A HUMAN MELANOMA CELL LINE (WMXRS-1), HYPERSENSITIVE TO IONISING AND ULTRAVIOLET (UV) RADIATION, AND UV-MIMETIC AGENTS

by

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The isolation of DNA repair-deficient cellular mutants has proved to be the most useful method for the evaluation of pathways for the repair of drug or radiation-induced damage to the genome. Characterisation of the particular repair deficiency has led in many cases to a detailed understanding of normal repair pathways, including the recent molecular cloning of six human genes associated with UV damage repair, and one x-ray repair gene. A heightened repair ability has been implicated as one mechanism of the intrinsic cellular radioresistance of many melanoma cell lines. Such cell lines may thus provide a relevant substrate for attempts to isolate further DNA repair genes.

Using the following novel screening method, the first human x-ray sensitive mutant has been isolated. Mutagenised human melanoma (MM96) cell colonies were replica plated between tissue culture plates. Three days after exposure to ionising radiation, cultures were incubated for 2hrs with the halogenated pyrimidine DNA precursor, BUdR (BRdU). Colonies which were synthesising DNA were identified immunochemically. Unlabelled, ie. non-proliferating, colonies were identified, and the corresponding colony on the unirradiated replica plate was localised, expanded and tested for radiosensitivity. Screening of 42,500 MM96 colonies yielded one IR-hypersensitive mutant, WMXRS-1 (Westmead Melanoma X-ray Sensitive-1), which is presently undergoing characterisation. In comparison to its parent, WMXRS-1 shows cross sensitivity to UV radiation and other agents known to be removed by the excision repair pathway (mitomycin C and cisplatin), but not to the 'x-radiomimetic' agent, bleomycin.

We conclude that this mutant phenotype should provide a powerful model for the study of both DNA repair after ionising radiation, and intrinsic cellular radioresistance, and is especially relevant given the considerable x-ray resistance of advanced melanoma.

RADIATION SENSITIVITY IN SCHIZOPHRENIA

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Abstract

Schizophrenia is a chronic brain disease that affects 1 in 100 members of the community. In recent years there has been an accumulation of evidence from neuro-imaging and histopathology that supports the neurodevelopmental hypothesis of schizophrenia. This theory suggests that there is a defect of neurodevelopment *in utero*, which manifests itself as schizophrenia in adult life. It is likely that the DNA processing mechanisms involved in gene rearrangements resulting in normal immunological function are also involved in rearranging the genome to achieve the complex neurological connections and pathways of the brain. Thus, if the DNA processing mechanisms are abnormal in Schizophrenia it is conceivable that this would be reflected in the lymphocytes. To investigate DNA processing abnormalities we used ionising radiation to damage EBV-transformed lymphoblastoid cells from schizophrenia patients and controls. These cells were followed for 24 hours after irradiation and their DNA content was periodically analysed using a FACSCAN.

Blood samples were collected from familial and sporadic schizophrenic subjects and an age and sex-matched control group. Familial subjects had a positive family history of schizophrenia in at least one first degree relative, while the sporadics had a negative history.

Lymphoblastoid cells were exposed to 100 rads of γ-radiation. At various times after irradiation aliquots were removed and the number of cells in G2 determined on the FACSCAN using propidium iodide as the stain.

Both familial and sporadic cell lines displayed abnormal responses to irradiation. At both the 8 and 12 hour time points the number of cells in G2 from schizophrenic subjects was significantly greater than for controls. However, the cells from familial subjects appeared to be more sensitive than the sporadics.

We are presently extending the numbers of subjects studied and varying the radiation doses as well as investigating other stress inducing agents.

CELL DEATH

by

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Abstract

After moderate doses of radiation, cells may show diminished proliferative capacity but preserved metabolic functions, a phenomenon referred to as reproductive "death". In rapidly proliferating and lymphoid cell populations, however, many cells actually die within a few hours of irradiation. This observable death takes the form of apoptosis.

Apoptosis differs from classical cell death or necrosis in morphology, biochemistry and incidence. The sequence of morphological changes suggests an active process and involves rapid condensation of the cell with surface convolution followed by separation of the surface protuberances to produce membrane-bounded globules in which organelles appear intact; these are quickly phagocytosed and digested by adjacent cells without associated inflammation. Biochemically, there is selective double-strand cleavage of nuclear DNA at the linker regions between nucleosomes. Protein synthesis is frequently, though perhaps not invariably, required, and several putative apoptosis-related genes have recently been identified. Apoptosis takes place continually in healthy animals, being involved in normal cellular turnover and in a variety of involutional processes. However, it is also induced by certain specific pathological stimuli. Necrosis, by contrast, is essentially degenerative in nature, and is invariably the outcome of severe cellular injury.

The induction of apoptosis by radiation in moderate doses and by other genotoxic agents such as chemical carcinogens can be viewed teleologically as a biologically useful, homoeostatic phenomenon; that is, altruistic cell suicide in the interests of the animal as a whole. Amplification of unrepaired genetic abnormalities in proliferating populations of cells is clearly undesirable. The unique sensitivity of interphase lymphocytes to radiation might be explained teleologically by the potential for mutant lymphoid cells to produce auto-immune disease. Phorbol esters have been shown to inhibit radiation-induced apoptosis; part of their tumour-promoting action may thus depend on their allowing mutations to persist.

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INCIDENCE OF RADIATION, HYPERTHERMIA, AND DRUG-INDUCED CELL DEATH (APOPTOSIS IN NEONATAL TISSUES

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ABSTRACT

This paper reports continuing experimental studies of the incidence of apoptosis induced by X-irradiation, hyperthermia, and some cytotoxic drugs on neonatal rat tissues. In the present study, a difference is shown in the presence and extent of apoptosis induced by these treatments in the kidney and testis of 4-day-old neonates. The immature animals were treated with one of the following protocols: 5Gy X-irradiation, immersion to the sternum in water at 42°C for 30 minutes, an ip. injection of adriamycin at concentrations from 2-20mg/kg of body weight, or an ip. injection of cycloheximide at concentrations ranging from 1.5-15mg/kg of body weight. Tissue from the kidney and testis was studied 6h later, using histology and DNA gel electrophoresis to identify and define the mode of cell death. In some cases, autoradiography was carried out after ^{3}H-thymidine incorporation was used to indicate S-phase in the cell cycle.

Apoptosis ("cell suicide", "programmed cell death") is thought to play a homeostatic role in normal neonatal tissues, by bringing about the selective elimination of cells with critical DNA damage, thus minimising the propagation of genetic errors. After all of the treatments, increased cell death by apoptosis, and not by necrosis, was observed. The extent of apoptosis induced in the two neonatal tissues was then quantified (see following Table).

Treatment	Presence of apoptosis (+++,++,+,0) (- = no result yet)		Cells in S-phase affected	
	Kidney	Testis	Kidney	Testis
Irradiation	+++	+++	Yes	No
Hyperthermia	+++	0	-	-
Adriamycin	+++	+	-	-
Cycloheximide	-	+	-	-

We conclude that, in these neonatal tissues, the kidney is more sensitive/responsive to the treatments than is the testis. Quantification of apoptosis in different neonatal tissues after anti-cancer treatments may provide new information about their effects, thus aiding the design and use of more effective treatment regimens.

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KINETICS OF RADIATION-INDUCED APOPTOSIS OF RAT SPERMATOGONIA

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Abstract

Spermatogonia are among the most radiation sensitive mammalian cells. Oakberg(1) demonstrated conclusively that physical deletion of spermatogonia, and not mitotic inhibition, was responsible for radiation-induced depopulation of the germinal epithelium. We have shown that apoptosis(2), not classical necrosis, is the mechanism effecting spermatogonial deletion during normal spermatogenesis and after x-irradiation (3). Here we report the detailed kinetics of radiation-induced spermatogonial apoptosis and present a mathematical model to fit the data for the B type spermatogonia. Normal and apoptotic spermatogonia were counted in histological sections 0 to 48 hours after 10 Gy x-irradiation (64 mature rats).

Following x-irradiation, there was a variable lag period of 6 to 36 hours after which all spermatogonia in a particular syncytium synchronously entered apoptosis. The median times of appearance and phase durations of early, intermediate and late apoptotic bodies induced in A_3 and B-type spermatogonia were:

		B type	A_3 type
Appearance:	early	12.6 ± 1.1h	8.1 ± 0.9h
	intermediate	14.5 ± 0.6h	12.7 ± 1.3h
	late	18.3 ± 0.2 h	13.6 ± 0.3h
Phase duration:	early	32.3 ± 2.0min	56.9 ± 15.8min
	intermediate	2.0 ± 0.1h	6.0 ± 0.3h
	late	4.9 ± 0.2h	8.5 ± 0.2h

What determines the lag in irradiated spermatogonia destined to undergo apoptosis is uncertain. Approximately 50% of spermatogonia undergo apoptosis between 6-12 hours and the remainder at a slower rate over the succeeding 24 hours; mathematical modelling suggests that the latter may be related to progression of initiated cells through the cell cycle.

The sequence apoptotic stimulus, lag and apoptotic response provides a structural reference which can be identified and quantified in tissues or cell populations in which apoptosis is investigated. Cellular targets by which susceptibility and sensitivity to apoptosis can be manipulated may be more precisely defined if biochemical and molecular data are correlated with the temporal sequence of events occuring in an individual apoptotic cell.

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ANTICANCER DRUGS INDUCE CELL DEATH BY APOPTOSIS OR NECROSIS

by

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Abstract

The mechanism of action of many cytotoxic drugs is known but precisely how drug treated cells die is unclear. Two modes of cell death, apoptosis and necrosis have been described and these can be distinguished biochemically.

The mode of cell death, after treatment with several classes of anticancer drugs was monitored in CCRF-CEM-F2 cells, by studying the appearance of DNA fragmentation patterns on agarose gels. After 48 hours treatment, while most drugs induced cell death by apoptosis which was characterised by DNA with the appearance of discrete oligonucleotides with multiples of about 200 b.p. 5-fluorouracil, mithramycin and heat caused cytotoxicity with random DNA breakdown which is characteristic of necrosis. The mode of cell death induced by cytotoxic drug treatment of 2 other human leukaemic T-cell lines (CCRF-HSB and MOLT.4) was also investigated for comparison. Cytotoxic drug treatment of CCRF-HSB caused a pattern of DNA degradation similar to CCRF-CEM-F2 except that methotrexate treatment also induced cell death by necrosis. However, MOLT.4 exhibited cell death by necrosis after all drug treatments investigated.

The effects of the RNA and protein synthesis inhibitors cycloheximide and actinomycin-D respectively were studied on drug treated cells. DNA fragmentation induced by dexamethasone, fluoredeoxyuridine and methotrexate was considerably reduced when cycloheximide was added together with the drug. However, cycloheximide did not influence DNA breakdown caused by 5-fluorouracil. Actinomycin-D did not inhibit DNA fragmentation caused by these results indicate that protein synthesis is required for apoptosis after drug treatment.

Differences in the pattern of cell death after drug treatments are now being utilised to explore differences in the metabolic trigger of the apoptotic process compared to necrosis. Since fluorodeoxyuridine induces apoptosis and fluorouracil treatment causes necrosis, a variety of modulators of drug action are being applied to cause specific biochemical perturbations which may influence these cytotoxic mechanisms. In addition, morphological studies are in progress to quantitate precisely the proportion of cells dying by apoptosis or necrosis in these drug treated cells.

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APOPTOSIS IN LYMPHOID CELLS

by

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Abstract

A variety of agents including glucocorticoid hormones, radiation, heat and chemicals activate a process in lymphoid cells called apoptosis or programmed cell death. This process has been characterized by well defined morphological changes that include margination of the cytoplasm, chromatin condensation and cell blebbing. In addition DNA is fragmented into oligonucleosome-sized fragments.

We have recently demonstrated that okadaic acid, a tumour promoter and inhibitor of protein phosphatases type -1 and -2A prevented apoptosis induced by either ionizing radiation or heat shock. Using 2-dimensional gel electrophoresis we have shown that apoptosis induced by both agents is accompanied by dephosphorylation of a limited number of proteins. Okadaic acid which prevented apoptosis also inhibited the dephosphorylation of some of these proteins.

Overexpression of different mRNAs was also observed in lymphoid cells undergoing apoptosis. One such mRNA was identified as that encoding a β-galactoside binding lectin which has recently been implicated in the control of passage of cells through the cell cycle.

The importance of these different observations to apoptosis will be discussed.

THE RELATIONSHIP BETWEEN MODE OF CELL DEATH AND SENSITIVITY TO RADIATION-INDUCED DNA DOUBLE-STRAND BREAKAGE FOR MOUSE HAEMOPOIETIC CELL LINES

by

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Abstract

Loss of metabolic function and subsequent disintegration, of mammalian cells that are reproductively dead following exposure to ionizing radiation, is generally a slow process that is dependent upon completion of mitosis. However, some cell types (e.g. resting lymphocytes) die rapidly, without undertaking mitosis, following irradiation. The latter cell types undergo a distinctive form of cell destruction termed "apoptosis"; whilst mitosis-related death appears to involve necrosis. A variety of evidence suggests that DNA double-strand breakage (dsb) is the critical radiation-induced lesion that ultimately leads to chromosome aberrations and mitosis-related death. It was therefore of interest to compare the sensitivity to DNA dsb of cells that undergo radiation-induced apoptotic death with cells that undergo necrotic death. For this purpose, a variety of mouse haemopoietic cell lines was used [1].

The mouse T lymphoma line STRij-4-2.2 (ST4), which undergoes rapid apoptotic-type disintegration after irradiation, showed extreme sensitivity to X-ray or DNA-associated ^{125}I decay-induced DNA dsb (7 ± 1 ^{125}I decays per clonogenic lethal event). Another T lymphoma line WEHI-22.1 (W22), which does not undergo rapid disintegration, was less sensitive (17 ± 1 ^{125}I decays) than ST4 cells but was still considerably more sensitive than the fibroblast-like line V79 (61 ± 2 ^{125}I decays) [2]. Further evidence of this type, for other haemopoietic cell lines, will be presented to examine whether cells that undergo apoptotic-type death are more sensitive to DNA dsb than are cells that undergo necrotic-type death.

The rapid disintegration of ST4 cells, induced by irradiation, was inhibited by incubation with phorbol esters or related diterpenes. Suggesting that de-phosphorylation events may be an important part of the apoptotic mechanism. Phorbol esters, such as PMA, were potent radioprotectors of ST4 (but not W22) cells. Data on the radioprotective ability of protein kinase C activators will also be presented.

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ß-radiation from tracer doses of ^{32}P induces massive apoptosis in a Burkitt's lymphoma cell line.

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Abstract

While investigating a possible role for protein phosphorylation in apoptosis, we added ^{32}P to cultures of a Burkitt's lymphoma (BL) cell line in which apoptosis was induced by various means. We found that, not only was apoptosis enhanced in the experimental cultures, but massive cell deletion by this process also took place in control cultures to which the isotope had been added.

The Epstein-Barr virus negative BL cell line BM13674 was being used in our investigations. This cell line is known to exhibit extensive apoptosis after exposure to a variety of stimuli, including mild hyperthermia (Harmon *et al.*, 1991), cancer chemotherapeutic agents and X-irradiation (Forster, unpublished observations).

The experimental protocol involved addition of ^{32}P, 0.05mCi/ml, to 48 hour cultures. Control cultures without isotope were kept remote from ^{32}P-containing flasks, which were placed in containment vessels made of 1cm thick perspex. Samples were taken at 2, 4, 10, 20 and 24 hours. They were processed for light and electron microscopy, and DNA extracted from them was subjected to agarose gel electrophoresis.

The rate of apoptosis scored by light microscopy was consistently higher in the cultures containing ^{32}P than in the controls; by 24 hours, it was approximately 20 times the control value, more than 90% of cells being affected. Identification of the apoptosis was confirmed by electron microscopy and by the development of a characteristic oligonucleosomal "ladder" on electrophoresis of extracted DNA.

Our findings have important consequences for researchers using ^{32}P (and possibly other radioactive isotopes) for biochemical studies. Radiation is known to induce apoptosis in many lymphoid and proliferating cell populations (Allan *et al.* 1988). Failure to take account of the occurrence of extensive cell death in experiments using radioactive tracers would inevitably lead to incorrect interpretation of the results.

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Photo-enhancement of the mutagenicity of 9-anilinoacridine derivatives related to the antitumour agent amsacrine

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The frameshift mutagenicity of the DNA intercalating drug proflavine is known to be enhanced by photoirradiation of bacterial cultures. To determine whether this phenomenon was also present in acridine-derived antitumour drugs, cultures of *Salmonella typhimurium* were exposed to the antileukaemia agent amsacrine and the experimental agent N-[2-(dimethylamino)ethyl]acridine-4-carboxamide dihydrochloride (acridine carboxamide) in the presence or absence of visible light. A small increase in mutagenicity was observed with amsacrine but not with acridine carboxamide. A series of analogues of amsacrine were then tested, and a striking relationship was found between the minimum drug concentration for mutagenicity and DNA binding affinity. In each case, photoirradiation was associated with a small increase in mutagenicity. Each of the compounds showing the photo-enhancement effect was capable of reversible one-electron oxidation. It is suggested that this oxidation occurs in bacteria, and that the DNA binding constant of the resulting acridine radical species will increase because of the extra positive charge. This increased DNA binding would be sufficient to explain the photo-enhancement of mutagenicity of these drugs.

THE CONVERSION OF EXCISION-REPAIRABLE DNA LESION TO MICRONUCLEI WITHIN ONE CELL CYCLE IN HUMAN LYMPHOCYTES

by

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Abstract

The human lymphocyte micronucleus (MN) assay is relatively insensitive to genotoxic agents that predominantly induce excision-repairable lesions such as adducts and abasic sites. In this study we have explored the possibility of using cytosine arabinoside (ARA) to convert excision-repairable DNA lesions to micronuclei (MN) within one cell cycle. The system consisted of human lymphocytes as target cells, the cytokinesis-block (CB) method for identifying cells that had completed one nuclear division only [1,2], and X-rays, methylnitrosourea (MNU) and ultraviolet light (UV) as mutagens. With each mutagen we have observed significant increments in induced MN in the cultures that had also been treated with ARA during G_1. The slope of the dose-response curves for induction of MN was increased by a factor of approximately 1.8 for X-rays and 10.3 for UV and significant MNU induction of MN was only achieved in the cultures treated with ARA. Furthermore, a 24-hour gap between mutagen exposure and the start of the assay did not abolish the increased mutagen sensitivity in the cultures treated with ARA. These observations suggested that the combined ARA and cytokinesis-block micronucleus (CBMN) method may enhance the detection of exposure to genotoxic agents that predominantly induce excision-repairable lesions. The ARA/CBMN method may also enable the measurement of base hydroxylation events following exposure to ionising radiation.

References

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RELATIONSHIP BETWEEN CHROMOSOMAL ABERRATIONS AT THE FIRST AND SECOND DIVISIONS FOLLOWING X-IRRADIATION, AND THEIR ASSOCIATION WITH CLONOGENIC SURVIVAL.

By

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Abstract

We have measured the types and frequencies of chromosomal aberrations in JU56 cells irradiated in G_1, and in cells irradiated in G_2, at the first and second division. To examine cells at the second division, irradiated cells were exposed to colcemid at the time of the first division, to prevent cytokinesis and the cell death which results from misassortment of acentric fragments, and the formation of chromosome bridges. The cells went back into interphase and after another round of replication underwent division again as pseudotetraploids.

For cells irradiated in G_1, and examined at the first division, it was found that the distribution of aberrations was not random; there was an excess of cells with no aberrations and of cells with multiple aberrations. The number of cells containing aberrations was insufficient to explain clonogenic cell death. Between the first and second divisions, there was a loss of cells with aberrations from the dividing fraction, despite the absence of cytokinesis. We interpret these results as indicating that some damage to DNA, resulting in chromosomal aberrations, or in a more sensitive fraction of cells which also contain aberrations, is unrepaired, and the unrepaired DNA is subject to digestion by nucleases during interphase, leading to loss of active genes and death, or failure to divide.

For cells irradiated in G_2, the number of cells with no aberrations is somewhat higher than the surviving fraction, and the frequency of cells without aberrations increases between the first and second division, although not so markedly as it does in cells irradiated in G_1. The frequency of aberrations is not reduced significantly, but there is evidence that new aberrations are produced between the first and second division.

It would appear therefore that the correlations observed between aberrations and cell death are only approximate, and that cell death following irradiation is not only due to loss or misassortment of genetic material when cells divide.

MICRONUCLEUS FREQUENCIES IN SPLEEN AND PERIPHERAL BLOOD LYMPHOCYTES OF THE MOUSE FOLLOWING ACUTE WHOLE BODY IRRADIATION

By

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and
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Abstract

The micronucleus (MN) frequency in spleen and peripheral blood lymphocytes of the mouse was determined using the cytokinesis-block MN assay, serially, for 14 days following acute whole-body ionising irradiation. The baseline MN frequency of spleen lymphocytes (7.86± 0.68, mean ± S.D.) was significantly ($p<0.001$) elevated when compared to that for peripheral blood lymphocytes (4.10 ± 0.53). Immediately after irradiation, the frequency of MN was increased in spleen lymphocytes (120.2 ± 9.4 for 1 Gy; 409.5 ± 38.4 for 2 Gy) and peripheral blood lymphocytes (78.0± 7.0 for 1 Gy; 200.2 ± 10.9 for 2 Gy). The MN frequencies in blood and spleen became approximately the same by day 14. During the 14 days after irradiation, the MN frequency in spleen lymphocytes, declined to approximately half of the value observed immediately after irradiation. The MN frequency in peripheral blood lymphocytes increased during the week after irradiation.

Study of isolated murine lymphocytes irradiated *in vitro* showed that the number of MN generated by a given dose of radiation was approximately 2-3 times greater than the number generated by *in vivo* irradiation.

These results suggest that the measurement of MN *in vivo* after irradiation can be used as an *in vivo* dosimeter. However, precise dosimetry is probably affected by factors such as kinetic changes in different lymphocyte populations and possibly by *in vivo* factors which influence sensitivity of cells to radiation.

DELAYED EFFECTS OF LOW LEVEL RADIATION

by

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Abstract

It is well established that chronic exposure to low level ionising radiation may lead to adverse effects - mainly to an enhanced risk of cancer, typically emerging ten to twenty years later, but also in the form of genetic changes.

The problem is to quantify this risk for humans in order to apply it to occupational and public radiation exposure limits, when typical exposures are so low that, in most cases, the resultant effects cannot be identified against a high background incidence from other causes.

We are forced therefore to take epidemiological data from more highly exposed groups and to make use of dose-effect models to extrapolate to the lower levels encountered in the occupational or public environment.

In recent years the doses associated with the relatively highly exposed groups of Japanese A-bomb survivors have been re-evaluated and are now considered to be significantly lower than the estimates of a decade ago. This result, together with changes in modelling, points towards a substantial reduction in occupational exposure limits.

SOME ASPECTS OF THE BACKGROUND AND RADIOBIOLOGICAL CONSEQUENCES OF THE CHERNOBYL ACCIDENT

by

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Abstract

The USSR has extensive reserves of coal, oil and gas, but for various reasons it was perceived some time ago that nuclear power provided the best means of meeting increasing energy demands.[1] The nuclear power development program was essentially a cooperation between the Soviet Union and Eastern bloc countries, although dominated by the USSR. There have been concerns about a number of aspects of the program, especially the pace of its implementation. The areas of concern included reactor design, construction and operating procedures, and the Chernobyl accident to a large extent reflected many of these problems. The Soviet response to the accident also met with considerable criticism, for example the failure to promptly provide information about the accident to the world press and to their own population, and delays in evacuation.

In June of this year the Soviet Academy of Sciences sponsored a conference on the Radiobiological Consequences of Nuclear Accidents. This paper will describe some of the data presented at the meeting, including both radioecological and radiobiological studies. The ^{137}Cs fall-out affected a large area of the best agricultural land in the USSR, and ecological monitoring stations in many parts of Europe recorded the consequences of the accident. The fact that these monitoring programs were set-up and operating in Western Europe provides a contrast of approaches to nuclear development. About 600,000 people, collectively referred to by the Soviets as "liquidators" were exposed to radiation as a result of the response to the accident. Levels of exposure varied widely and there is considerably uncertainty as to the extent of the effects on health. A number of presentations highlighted evidence for heterogeneity of radiosensitivity amongst individuals. The Soviets have established a data base of almost 576,000 individuals with an estimated average exposure of 0.125 Gy. Compilation of the data base is not complete; a number of the liquidators have yet to be traced.

Reference

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CHERNOBYL - EVALUATION OF THE CONSEQUENCES

by

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Abstract

It is now openly admitted by the Soviet authorities that the extent of the problem that existed in Byelorussia and other neighbouring regions in the days and weeks immediately following the incident at Chernobyl was not revealed to the public, but rather it was deliberately concealed. When it became evident that the problem was not exclusively a Soviet one and would not be solved by internal effort alone attitudes changed.

While attending a recent conference on "Low Dose Radiation Exposure" in Moscow, 4th -7th June 1991 I was invited with several other foreign scientists to a special committee of the Supreme Soviet on Chernobyl. This committee was made up of deputies of the Supreme Soviet and eminent scientists from a number of different disciplines to provide an integrated approach in determining "the truth". These disciplines included, radiation ecology, biology, genetics, geoecology, radioactive waste disposal, legislation and physical causes. The effort was coordinated from the Supreme Soviet Building in Moscow and involved approximately 30 separate directions with ad hoc committees established in various localities in the regions worst affected. Members had to contend not only with the situation that existed but also with the historical impact. Unlike the situation in France the nuclear industry in the Soviet Union was derived from atomic nuclear bomb exploration. It was dominated by the military and did not have the direction of industry.

These and other aspects of the Chernobyl incident will be discussed.

BIOLOGICAL DOSIMETRY AFTER RADIATION ACCIDENTS

by

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Abstract

In the absence of physical estimates from personal monitors or other sources, biological dosimetry may be the only means of reliably estimating the magnitude of an accidental whole body exposure to external radiation. The frequency of unstable chromosomal aberrations in circulating blood cells represents the most sensitive biological measure of radiation damage and development of the basic technique over more than 20 years has resulted in its routine application in radiological protection.

The main limitations of the method are threefold:

1) The lower limit of sensitivity - about 100 mGy for a whole body exposure to gamma rays.

2) The time to obtain a result - normally about 3 man-days is required for the microscope analysis.

3) The problems in interpretation presented by partial body exposure, either to external radiation or to an internally incorporated radionuclide.

Despite these limitations the practical value of the technique was graphically demonstrated in the immediate aftermath of the major accidents at Chernobyl (1986) and Goiania (1987). Initial, very approximate biological dosimetry estimates carried out on several hundred people proved invaluable in identifying those who had received significant exposure. Further more detailed analysis assisted greatly in the medical management of those most severely irradiated.

MUTAGENIC AND CARCINOGENIC EFFECTS OF LOW LEVELS OF IONIZING RADIATION

by

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Abstract

Until relatively recently, it was difficult to suggest any convincing theoretical or molecular basis for assuming that low levels of ionizing radiation were not capable of exerting mutagenic effects (and hence potential carcinogenic effects) at doses very close to zero. Indeed, the concept that mutagenesis is an intrinsic property of cells which depends upon misrepair of radiation-damaged DNA by individual cells within an exposed population implies that DNA repair *per se* can not be viewed simply as a protective mechanism. On the contrary, DNA repair has to be viewed as *causative* in mutagenesis rather than being responsible for an antimutagenic effect. The validity of this argument depends upon a widely-held assumption that so-called "spontaneous" mutagenic events are relatively rare and are in turn associated with relatively rare types of DNA lesions of a different sort from those induced by radiation exposure. The recent realisation that oxidative damage generates lesions which are (i) very similar if not identical to those induced by ionizing radiation and (ii) not at all rare, arising at a frequency several orders of magnitude greater than the comparable lesions generated by low doses of radiation, has provided a scientifically respectable explanation for radiation-induced mutagenesis (and probably also the initiation stage of carcincogenesis) being less than linear at low doses. The implications of these findings for estimating low level radiation effects in humans are not without significance.

RADIATION HORMESIS

by

J.K. Brown

Abstract

Hormesis is a general phenomenon in which exposure to low levels of toxic agents stimulates natural physiological defense mechanisms in a manner which benefits health and increases lifespan, growth and development, fertility, immune competence, resistance to infection and possibly protects against ionising radiation. A variety of prokaryotic and eukaryotic cells exhibit induction of hormesis by a wide range of physical and chemical agents, employing many biological endpoints.

Over the last decade there has been increased interest in studies of the induction of DNA repair systems occurring in the cell after exposure to very low doses of mutagens such as ultra violet rays, alkylating agents, heat, oxidising agents and ionising radiation. By these mechanisms the cell (and organism) respond protectively to toxic insults from the environment.

A radiation hormesis effect was first observed in human lymphocyctes *in vitro* in 1984. Cultured lymphocytes exposed to very low levels of tritiated thymidine were observed to become resistant to the induction of chromosomal damage by subsequent high doses of X-rays. The radio-adaptive response has been subsequently shown to occur in cells pre-exposed to very low doses of low LET radiations such as β, and X-rays but has not, as yet, been confirmed experimentally for high LET radiations.

Typically the first "conditioning" dose is of the order of 0.01Gy (1 rad) and the second "challenging" dose > 1Gy (100 rads). The first dose, whether it be ionising radiation or a chemical mutagen, is too low to produce any discernible cytogenetic damage. Agents producing similar effects to X-rays also induce an adaptive response, such as radiomimetic bleomycin (double strands breaks), mitomycin (cross links in DNA) or hydrogen peroxide (oxygenated free radicals). The magnitude of the reduction in chromosomal damage can be up to 50% but is variable between donors depending on various physiological factors such as the concentration of plasma cortico-suprarenal hormones rather than being of genetic origin.

The radio-adaptive repair mechanism commences to operate about 4 hours after exposure to the conditioning dose and lasts for three cell cycles in human lymphocytes. That inducible proteins are involved in the repair mechanism has been demonstrated by the action of the protein inhibitor cyloheximide and the electrophoretic isolation of several new proteins from cultured lymphocytes after exposure to 0.01Gy X-rays. The hormetic effect can also be inhibited by 3-aminobenzamide indicating the likely involvement of poly(ADP-ribose) polymerase, an enzyme involved in the repair of double strand breaks in DNA.

The possible consequences of radiation hormesis in radiological protection guidelines will be discussed.

CLINICAL APPLICATIONS OF RADIATION

by

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Abstract

This lecture will be divided into three parts and will attempt to address the following issues:

1. The clinical role of radiotherapy in comparison to the role of other forms of anticancer treatment (surgery, chemotherapy).

2. An overview of the clinical use of radiotherapy. This section will be divided into four parts, as we look at the varied roles of:

   a) external beam therapy (photons, electrons) using either standard or large fields
   b) interstitial implantation
   c) intracavitary radiotherapy
   d) the clinical use of radioactive isotopes for patients with thyroid cancer and polycythaemia rubra vera.

3. Are there any recent trends in the clinical usage of radiotherapy in Australia? The declining role of radiotherapy in the treatment of superficial skin cancer will be mentioned, as will the increasing role of radiotherapy in the treatment of patients with early stage breast cancer following lumpectomy.

DOES BORON NEUTRON CAPTURE THERAPY REGRESS CANCER ?

by

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Abstract

In recent years a range of animal and human studies have been carried out on the uptake of boron compounds by neoplasms and their regression by Boron Neutron Capture Therapy (BNCT). Results will be presented for experiments with mice, rats rabbits, pigs, dogs and human patients from laboratories in Europe, USA, Japan and Australia.

Most of the tumour xenograft models have centred upon melanoma and glioma cancers. In addition, the majority of the biodistribution studies have involved the use of Borocaptate Sodium (BSH) and Boronophenylalanine (BPA) which have shown the most promise for BNCT. Other more recently developed compounds,which have also been trialed in rodent species, are BSSB and BOPP.

Tumour to blood boron ratios achieved after administration of BSH are in the order of 2-3. This ratio is improved to approximately 8-12 if BPA is administered. Tumour to normal tissue boron ratios for both BPA and BSH are in the range of 3-5.

Where NCT has been employed, either thermal and epithermal neutron beams have been utilised. The therapeutic boron concentration required in the tumour is approximately 15-30 ppm. In all experiments using the thermal neutron beam, in conjunction with the boron compounds, have resulted in complete regression in approximately 60% of cases. In some models this success is as high as 80%.

This paper will review specific models, boron biodistributions attained as well as preclinical and clinical results achieved by treatment with BNCT.

References

[1] Proceedings of the "Fourth International Symposium for Boron Neutron Capture Therapy" and papers therein; Sydney, December 1990.

Acknowledgments

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NEUTRON SOURCES FOR BORON NEUTRON CAPTURE THERPAY

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Abstract

Currently only thermal neutron capture therapy is practiced in Japan, where reactors at Kyota University Research reactor Institute and Japanese Atomic Energy Research Institute provide limited access for the intra-operative treatment of high grade brain tumours and cutaneous melanoma. Epithermal beams are available at Brookhaven National Laboratory and Massachusetts Institute of Technology in the USA and installation is nearing completion at the JRC Reactor at Petten, The Netherlands. Clinical trials with epithermal beams are expected to begin in 1992.

In Australia, two reactors are available for NCT. Thermal NCT is carried out on small rodents in Moata, the 100kW Argonnaut reactor. A thermal patient NCT facility could be installed at HIFAR, a 10 MW reactor of the DIDO class. However, clinicians would prefer that an epithermal beam be available for the treatment of deep-seated tumours. Our design considerations to date indicate that a therapeutic epithermal beam from the HIFAR 10H facility is feasible and further calculations and experiments are underway to confirm this position.

We estimate that an epithermal neutron flux of 0.5 10^9 n $cm^{-2}s^{-1}$ will be available. On this basis, the outpatient treatment schedule would be 5 dose fractions, with bilateral exposures of 20 min each, for whole brain treatment for high grade brain tumours.

Measurements at the 10H beam hole with the existing a 2 cm square collimator indicate a thermal flux of 1.0 E09, a useful epithermal flux of 0.3 E09 and gamma dose rate of 70 Gy/h. By maximising the possible solid angle at the core, using an Al spectrum shifter and liquid argon gamma absorber, and reactor power of 15 MW, the desired epithermal flux should be achievable (2). The liquid argon filter is critical for the reduction in gamma ray dose to acceptable levels while still transmitting the epithermal neutrons.

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THE ROLE OF HEAVY WATER IN DOSE-DEPTH ENHANCEMENT IN THERMAL NEUTRON CAPTURE THERAPY

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Abstract

Following earlier research in the biological and neutron transport effects of heavy water, heavy water has been used in Japan to enhance the limited dose-depth distribution of thermal neutrons in the treatment of glioblastoma by Neutron Capture Therapy. Evidence to date [1] indicates that thermal NCT is efficacious for the more superficial tumours for which an adequate dose can be delivered to the posterior tumour without exceeding normal tissue tolerance at the surface of the brain. Prof Hatanaka has now treated several Australian patients with high grade brain tumours. Heavy water has been administered taken orally for several days prior to NCT, and concentrations of about 20% have been achieved. The effect of this degree of light water replacement is to increase the neutron transmission to the posterior tumour to a greater degree at depths exceeding 4 cm^2.

Heavy water concentrations are being determined by FTIR analysis of urine and blood samples [3] for several patients treated by NCT, and results will be available for discussion.

References

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3 N Blagojevic, B J Allen, K J Gaskin, L A Baur, Determination of total body water by fourier transform infrared analysis, Australas Phys Eng Sci Med 1990, 13,110-116

Acknowledgements

Work supported in part by the Department of Trade, Industry and Commerce.

THE EFFECTS OF THREE BIOREDUCTIVE DRUGS ON CELL LINES SELECTED FOR THEIR SENSITIVITY TO MITOMYCIN C OR IONISING RADIATION

by

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Abstract

Hypoxic cells within tumours are known to limit the efficacy of radiotherapy. Bioreductive and radiosensitizing drugs are under development in order to improve tumour therapy by sensitizing tumour cells to either radiation or cytotoxic chemotherapy. One process by which hypoxic cells can be selectively killed is to use the reductive metabolic pathways which can be readily exploited under poorly oxygenated conditions.

The mechanisms of action of three different classes of bioreductive compounds have been investigated *in vitro* using cell lines selected for their sensitivity to DNA damaging agents. Two groups of cell lines were used; MMC cells (1) derived from CHO-KI cells exhibit aerobic sensitivity to Mitomycin C (MMC) and *irs* cells (2) cloned from V79 cells which show sensitivity to ionising radiation. The sensitivity of both groups of cells to the bioreductive compounds Mitomycin C, RSU-1069 and SR4233 was assessed under both aerobic and hypoxic conditions.

No difference in aerobic or hypoxic sensitivity to MMC was observed for CHO-KI or two MMC sensitive cell lines. However, the MMC resistant cell line (MMC^r) was 10 times more sensitive under hypoxic conditions. In contrast differential toxicities of between 3 and 30 have been observed for CHO cells treated with RSU-1069 and SR4233. These results suggest that MMC^r cells lack, or are deficient in the enzymes responsible for activating MMC under aerobic conditions. In contrast, RSU-1069 and SR4233 treatment of V79 and *irs* cells showed selective toxicity towards hypoxic cells. For both RSU-1069 and SR4233 the hypoxic toxicities were similar in V79 and *irs* cells but in air, the radiation sensitive cells were up to 10 times more sensitive than wild type cells. These differences indicate that the *irs* cells may have a reduced capacity to cope with the drug induced damage compared to V79 cells.

Mechanisms involving drug transport, activation and elimination or repair of drug induced lesion may each influence the levels of drug sensitivity observed in the mutant cell lines.

References

[1] C.N. Robson et al., Cancer Res. (1985) 45, 5304.

[2] N.J. Jones et al., Mutat. Res. (1987) 183, 279.

HISTOLOGICAL EXAMINATION OF MELANOMA XENOGRAFTS IN THE NUDE MOUSE MODEL: PRE AND POST NEUTRON CAPTURE THERAPY

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3 Biomedicine and Health, Ansto PMB 1 Menai NSW 2234

Abstract

Following the inoculation of 1.5 million Harding Passey melanoma cells in the thigh, subcutaneous (sc) murine melanoma xenografts were grown in the nude mouse model. Mice received 12 mg of enriched boron-10 boronophenylalanine (^{10}BPA; NCT mice) or no BPA (neutrons only mice) and the tumours exposed to a neutron fluence of 10^{13} n cm^{-2} four hours post ip injection (1). Tumours in the neutrons only mice regressed but all recurred, showing a growth delay of about 3 weeks. However, only 1 of 8 NCT mice showed short term recurrence and 1 of 7 long term recurrence.

Three of the NCT mice lived in excess of 300 days before being sacrificed. The irradiated tumour beds showed a dense black mass still visible under the skin but no tumour growth was observed. Sections of the residual NCT tumours, of tumours treated with neutrons only and of untreated tumours were made and stained with haematoxylin and eosin (H&E). No mitoses were observed in the NCT sections, whereas these are evident in all untreated tumours. However, pleomorphic cells were observed in close proximity to capillaries and surrounded by cells with the appearance of histiocytes containing brown pigment. These cells are being examined by other techniques such as electron microscopy to determine whether or not they are melanoma cells.

References

1 B J Allen, S Corderoy-Buck, D E Moore, Y Mishima, M Ichihashi, Local control of murine melanoma xenografts in nude mice by neutron capture therapy; Neutron Capture Therapy for Cancer, Ed B J Allen, D E Moore, B V Harrington, Plenum Press NY, in press.

Acknowledgement

Work supported in part by the Government Employees Assistance to Medical Research Fund.

THE ANSTO BODY PROTEIN MONITOR

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ABSTRACT

The first Body Protein Monitor (BPM) to operate in Australia[1,2] was fabricated at the Lucas Heights Research Laboratories in 1985. In collaboration with The Children's Hospital, Camperdown, this instrument was successfully used in a study of protein deposition in children with cystic fibrosis[3]. Other clinical studies followed of end-stage renal failure[4,5] and the effects of aortic surgery[6]. Current projects include phenylketonuria, anorexia nervosa, liver disease and transplantation, synthetic growth hormone, intravenous fat emulsions, and paediatric arthritis. Some six Sydney hospitals are involved with these projects. The ANSTO prototype ANSTO BPM is now located in the Nuclear Medicine Department of the Royal North Shore Hospital.

The widespread application of the BPM and its acceptance by Sydney medical specialists as a vital element in clinical trials led to the design and fabrication of a commercial model. This unit has been sold to the University of Texas Medical Branch, and has been designed to study obese subjects. The BPM features a computer controlled table operation which allows all subjects to be measured to the same statistical precision. The Am Be neutron sources, supplied by the customer, can be turned on and off by virtue of rotating shield and collimator drums. Other features include the low background around the monitor, aluminium construction, top and bottom neutron sources, 4 high efficiency 10 x 10 x 20cm NaI detectors and the 7 minute counting time. There is also a provision for a bismuth germanate detector for the direct measurement of whole body chlorine. The IBM PC computer system is based on the Canberra S100 multichannel analysis card and Microsoft Windows Software operation.

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RADIOPHARMACEUTICALS

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Abstract

1991 is an exciting year for radiopharmaceutical chemistry and its applications to Nuclear and Molecular medicine in Australia. The National Medical Cyclotron (NMC) located at the Royal Prince Alfred Hospital (RPAH) Campus adjacent to the University of Sydney, was delivered in April. The first beam tests occurred in July while acceptance testing was scheduled for September 1991. The NMC is a unique facility in that it has objectives to produce short-lived commercial radiopharmaceuticals for marketing and distribution; the production of positron-emitting radionuclides for clinical PET at RPAH on demand; research and development of new investigational radiopharmaceuticals; research and post-graduate education.

Where appropriate, the Ansto reactor is utilised to produce radionuclides for development of radioimmunotherapeutic pharmaceuticals, or radiation synovectomy. The introduction of microdosimetry is a new dimension in radiopharmaceutical development at Ansto.

Recent developments in the radiopharmaceutical sciences relevant to this new Australian technology will be highlighted. Attention will focus on molecular radiopharmaceuticals using short-lived radionuclides and their relevance to improved health care delivery.

DESIGN OF THE ACCESS CONTROL AND RADIATION MONITORING SYSTEM FOR THE AUSTIN HOSPITAL POSITRON EMISSION TOMOGRAPHY CENTRE

by

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Abstract

A Positron Emission Tomography (PET) Centre is being established at the Austin Hospital, Melbourne[1]. The Centre will consist of a 10 MeV cyclotron, radiochemistry laboratories, PET scanner and image analysis laboratories. The equipment will be located immediately adjacent to patient and publicly accessible areas, necessitating strict and rigorous control and monitoring of the emitted radiation.

The cyclotron vault has been designed to shield against neutrons (energy spectrum up to 8 MeV) and gamma radiation to limit the external radiation dose levels to less than 1 milliSievert per year. Radioactive gases produced for patient inhalation studies ($C^{15}O$, $C^{15}O_2$, $^{15}O_2$) will be transferred in shielded lines and stored in patient delivery and waste gas disposal systems. An automated radiation monitoring system has been planned for continuous monitoring of neutron and gamma radiation levels in the vault, and for gamma radiation monitoring elsewhere. The system will also include air monitoring in the vicinity of the radioactive gas delivery and disposal system.

The vault and radiochemistry laboratories have been categorized according to the National Council on Radiation Protection and Measurements guidelines[2] for access control to radiation areas. An access control system incorporating visual alarm systems, signs, barriers and interlocks has been designed for the safe operation of the Centre.

References

[1] Egan, G.F., "Status of the Austin Hospital Positron Emission Tomography Centre", J.Aust.Phys.Eng.Med.14:42-47,1991

[2] NCRP Report #88, "Radiation Alarms and Access Control"

GENERAL INFORMATION

GENERAL INFORMATION

CONFERENCE VENUE

The conference will be held in the AINSE Theatre (Institute building), Lucas Heights see map page 59, from Wednesday 2nd October to Friday 4th October 1991.

PAPERS

The co-operation of session chairmen and speakers is sought in keeping presentation strictly to the scheduled times.

Timing

Green light shows for presentation of paper,
Warning lights show when 5 & 2 minutes are remaining,
Red light shows when presentation time has expired,
Discussion time of 5 minutes is then allowed by the Chairman.

Video projection with computer interface is available, if required.

Slides

Authors using 35 mm slides in conjunction with their talk are requested to place their slides in the projector magazine during the break preceding the session in which the paper is scheduled.

Poster Sessions

The poster sessions will be held in the ANSTO Technology Park Building, ref. map page 59, at the scheduled time (see program). Posters should be set up before the first poster session commences and removed after the Thursday session. Posters should be prepared before arrival at the conference in accordance with the guidelines previously provided.
Authors are expected to be in attendance by their posters throughout the sessions.
Materials for setting up posters will be available.

ACCOMMODATION

For participants distant from Sydney, accommodation has been arranged at Stevens Hall, Lucas Heights adjacent to the Institute Building, outside the main gate, in accordance with advised requirements. The Institute will make payment directly to the management for room only charges. Participants should make personal arrangements to pay cash for breakfast charges. Participants are requested to vacate their rooms by 9.00 am Friday, and to leave luggage in the room marked "luggage" adjacent to the theatre foyer. Please leave room keys in the reception office.

MEALS

Breakfast

For Stevens Hall residents, breakfast will be served in the ANSTO canteen from 7.30 am.
Participants should make arrangements to pay cash for breakfast charges.

Lunches

Conference Lunch - Wednesday 2nd October
Lunch for all participants will be held in the Stevens Hall dining room during the scheduled lunch period (ref. program).

Thursday and Friday 3rd & 4th October
Lunches may be purchased from the ANSTO canteen.

Evening Meals

BBQ Evening Meal - Wednesday 2nd October
Gather by ANSTO swimming pool.

Conference Dinner - Thursday 3rd October
Sutherland District Trade Union Club, Southern Cross Lounge, 57 Manchester Road (nr. Kingsway), Gymea.
Speaker: Dr. R. Cooper (University of Melbourne).

Note: No other evening meal services will be available at Lucas Heights.

IMPORTANT

Evening Meal - Tuesday 2nd October
Please advise the Conference Secretary prior to the Conference if you require an evening meal on this evening. It is essential to book for this meal.

TRANSPORT

Transport from Sydney Airport - Wednesday 2nd October
An ANSTO bus will leave the Australian Airlines Terminal (Sydney Airport) for Lucas Heights at 9.25 am. Kindly give your name to the driver when entering the bus. If any difficulty is experienced in locating the bus, the Australian Airlines Commonwealth Car Desk will advise details of its precise location.

Request to Participants Arriving on ANSTO Bus from Airport
As time will be limited, participants are asked to proceed directly to the AINSE Theatre and not to Stevens Hall. Luggage may be left in a room adjoining the theatre foyer marked "luggage". Keys may be picked up from Stevens Hall reception at any time during the day.

Transport from Sydney Airport (excluding Special Arrangements for Wednesday 2nd October)

Taxis are available from the airport to:-

Lucas Heights,
Sydenham Railway Station - then train to Sutherland Station,
Sutherland Railway Station - then bus to Lucas Heights(Friday only), or taxi
(ref. bus timetable below)

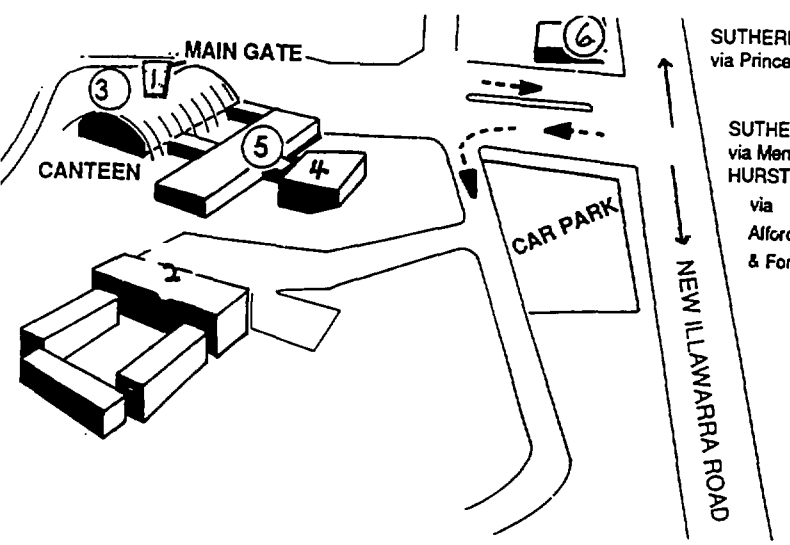
Note: Bookings must be made for all transport listed below - through AINSE, as transport is only provided if demand exists.

Monday - Friday only	
Depart Lucas Hts. for Sutherland	Depart Sutherland for Lucas Hts.
6.15 am	6.50 am
7.35 am	7.55 am
8.30 am	8.15 am
9.35 am	9.05 am
10.35 am	10.05 am
11.30 am	11.10 am
12.30 pm	12.00 pm
1.00 pm	1.10 pm
2.15 pm	1.30 pm
3.35 pm	2.45 pm
4.50 pm	
6.00 pm	
8.30 pm	

Buses from Lucas Heights leave from outside ANSTO Main Gate

Buses from Sutherland leave from outside Post Office, Flora Street.

LUCAS HEIGHTS N.S.W.



TRANSPORT

Transport from Lucas Heights to Sydney Airport - Friday 4th October

Transport will be arranged to take participants to Sydney airport before the cyclotron visit if required and after the conference concludes. Please place your name on the list provided, on the noticeboard outside the theatre if you require this service.

Participants leaving Stevens Hall - Friday 4th October

Participants are requested to vacate their rooms by 9.00 am. Luggage may be left in the room marked "luggage" adjacent to the AINSE theatre foyer. Room keys may be left in the Stevens Hall reception office.

TELEPHONE MESSAGES

Telephone messages will be taken for conference participants on:-

543-3411	543-3436
(AINSE)	(AINSE)

All enquiries concerning the Conference arrangements should be directed to:-

Joan Watson,
Conference Secretary,
A.I.N.S.E.,
Private Mail Bag 1,
MENAI NSW 2234

Phone: 543-3411 or 543-3436

L I S T O F P A R T I C I P A N T S

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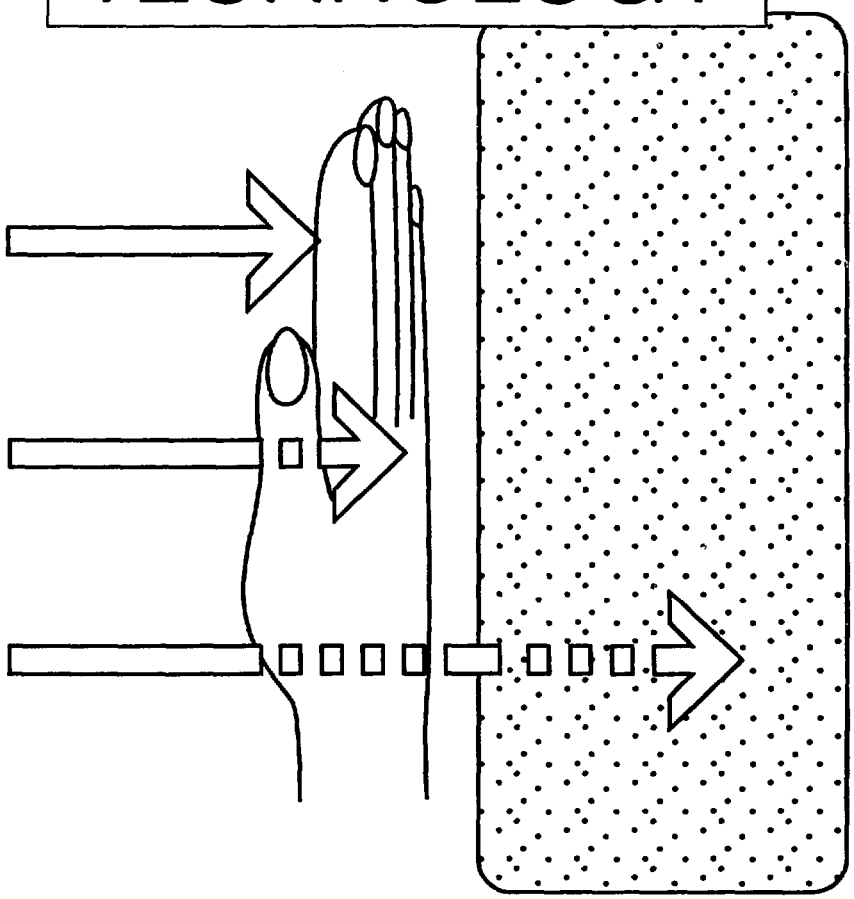

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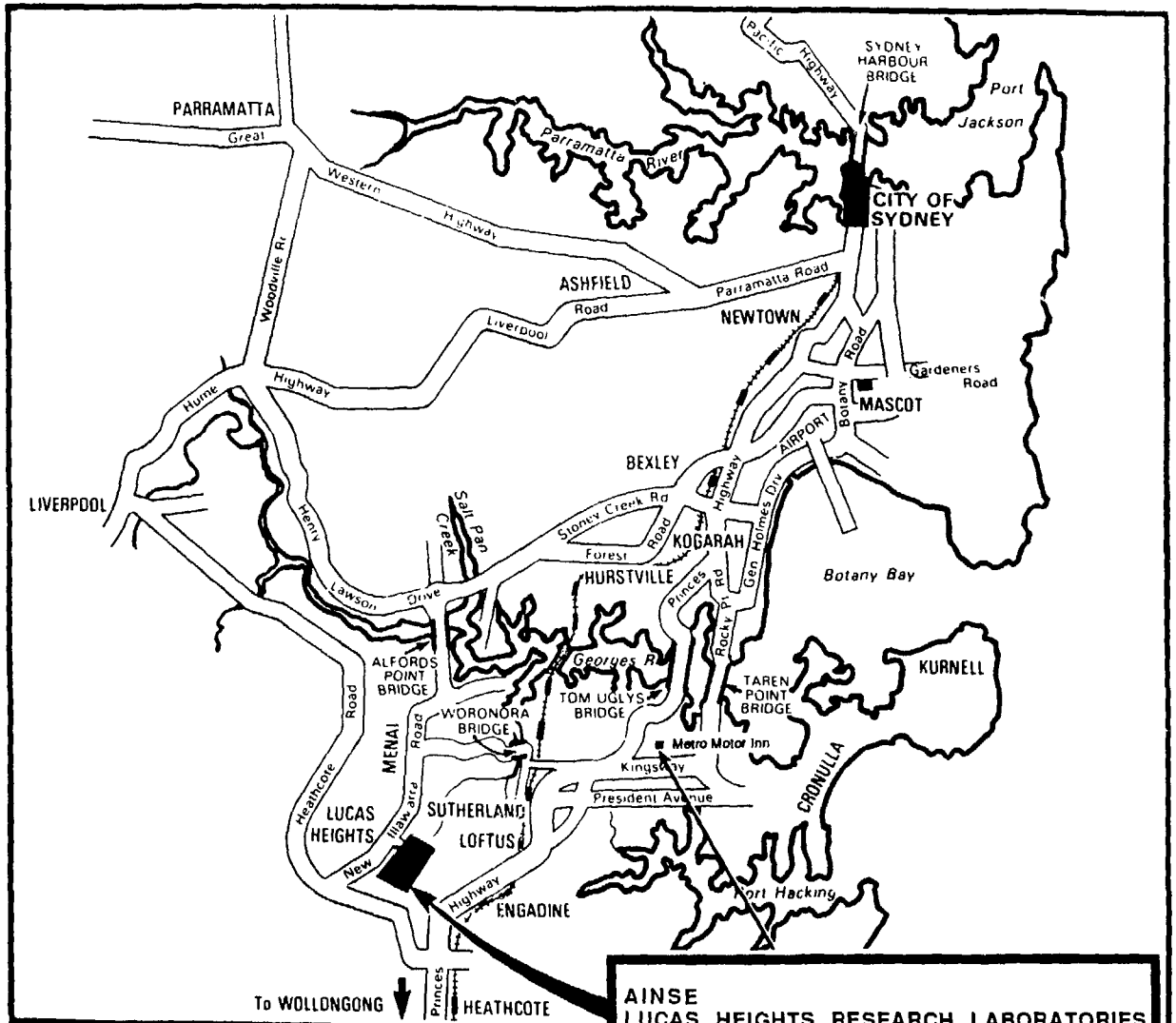

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