

## THE EFFECTS OF 3-AMINOBENZAMIDE AND APHIDICOLIN ON X-RAY-INDUCED CHROMOSOMAL ABERRATIONS IN CYCLING AND NON-CYCLING DOWN LYMPHOCYTES

Awadhesh Nandan Jha<sup>1,3</sup>, Tikaram Sharma<sup>1</sup> and Gokaran Prasad Katiyar<sup>2</sup>

### ABSTRACT

Aphidicolin (APC) and 3-aminobenzamide (3AB) were used to elucidate the role of DNA repair processes in the increased production of X-ray-induced chromosomal aberrations in lymphocyte cultures from Down syndrome patients (Downs), compared to normal controls. In the phytohaemagglutinin (PHA)-stimulated condition (G1 phase) the normal controls showed a significant increase in the yield of dicentrics, but not acentrics. In the Down cultures, the stimulated (G1) compared to unstimulated (G0) cells showed a significant increase of dicentrics as well as acentrics. 3AB potentiated the yield of aberrations only in stimulated lymphocytes of the groups, more in normal controls than in Downs. The potentiating effect of APC, in contrast, was more pronounced in the unstimulated compared to stimulated condition. In the stimulated condition, like 3AB, the potentiating effect of APC was greater in normal controls. The differential processing of X-ray-induced genetic damage in peripheral lymphocytes from Downs is sensitive to the presence of APC and 3AB. It is likely that both DNA polymerase  $\alpha$  and poly (ADP-ribose) polymerase have inherent differential behaviour in PHA-responsive Down lymphocytes.

### INTRODUCTION

Peripheral blood lymphocytes from Down syndrome patients (Downs), when exposed to ionizing radiations under *in vitro* conditions, show a higher incidence of

---

<sup>1</sup> Cytogenetics Laboratory, Centre of Advanced Study in Zoology, Banaras Hindu University, Varanasi-221 005, India.

<sup>2</sup> Department of Pediatrics, Institute of Medical Science, Banaras Hindu University, Varanasi-221 005, India.

<sup>3</sup> Present address: Brixham Environmental Laboratory, ZENECA Limited, Brixham, Devon TQ5 8BA, UK. Send correspondence to A.N.J.

chromosomal aberrations, especially asymmetrical exchanges, when compared to normal, diploid controls (Sasaki *et al.*, 1970; Preston, 1981; Leonard and Mertz, 1983; Morimoto *et al.*, 1984). Studies on induction of chromosomal aberrations by ionizing radiations in G1 and S phases of the cell cycle (after mitogen stimulation) have also shown that lymphocytes from Downs are more sensitive when compared to normal controls (Leonard and Mertz, 1983; Shafik *et al.*, 1988).

The mechanisms for increased chromosomal radiosensitivity in Down lymphocytes are not clearly defined. Various explanations have been given to explain this phenomenon, such as an altered rate of DNA repair efficiency (Preston, 1981) or competition between hypothetical 'error-free' and 'error-prone' repair systems that generate chromosomal aberrations (Countryman *et al.*, 1977), apart from being an inherent defect for the repair of induced DNA damage *per se* (Shafik *et al.*, 1988).

Aphidicolin (APC) is a tetracyclic diterpenoid antibiotic isolated from *Cephalosporium aphidicola*, and other fungi. It inhibits the growth of eukaryotic cells by inhibition of DNA synthesis. This action involved specific inhibition of DNA polymerase  $\alpha$  complex (Ikegami *et al.*, 1978; Huberman, 1981) which in addition to normal DNA synthesis also plays role in DNA repair processes (Natarajan *et al.*, 1982). 3-Aminobenzamide (3AB) is considered to be a potent inhibitor of poly (ADP-ribose) synthesis in mammalian cells as it competes with nicotinamide adenine dinucleotide (NAD) for reaction with poly (ADP-ribose) polymerase (Purnell and Whish, 1980). Poly (ADP-ribose) polymerase is activated by induced DNA-strand breaks and thus the synthesis of poly (ADP-ribose) usually increases during the cellular response to DNA damage (Berger *et al.*, 1979; Benjamine and Gill, 1980). The inhibitors of poly (ADP-ribose) polymerase, such as 3AB, have been found to prevent DNA strand rejoining and interfere with cell survival after DNA damage (Durkacz *et al.*, 1980). The use of APC and 3AB, as DNA repair inhibitors could provide additional information pertaining to the different mechanisms of aberration formation in the irradiated lymphocytes of Downs. We therefore examined the modifying effects of APC and 3AB on the X-ray-induced chromosomal aberrations in peripheral blood lymphocytes of Downs and compared them to normal controls. The studies were performed at two stages (G0 and G1) of the cell cycle, where presumably only repair synthesis takes place after primary lesions are induced in DNA.

## MATERIAL AND METHODS

Peripheral blood samples were collected in heparinized syringes from Downs and normal control individuals (three from each group). For irradiation in the G0 stage of the cell cycle, blood samples were X-irradiated with 2 Gy using a 110 kV Picker's therapeutic unit (dose rate 1 Gy/min) within 2 h of collection whereas G1 stage irradiation

was performed 10 h after the cultures were grown in phytohaemagglutinin (PHA) supplemented complete medium. Immediately after irradiation, the samples were treated with 3AB (3 mM) or APC (5 µg/ml) and incubated 2 h at 37°C. After incubation, the blood samples (G0 stage) or the cultures (G1 stage) were washed thrice with fresh culture medium and then allowed to grow in complete medium. The cultures were established using 0.30 ml of whole blood to 5 ml of RPMI 1640 medium, supplemented with 20% heat-inactivated human AB<sup>+</sup> serum. Antibiotics (1000 IU penicillin and 500 µg streptomycin/L), L-glutamine (2 mM) and Bromodeoxyuridine (BrdU, 5 µg/ml) were also added to the medium. All treatments were simultaneously given in blood samples of Downs as well as normal controls. The cultures were fixed 56 h after the addition of PHA, irrespective of the time of the treatment, following a 3 h colcemid block. Standard methods, as described earlier (Jha and Sharma, 1991) were followed for fixation of the cells and preparation of slides. The prepared slides were processed for fluorescent plus Giemsa (FPG) staining by the method of Goto *et al.* (1975), with some modifications. A minimum number of 100 first division cells were analysed for each treatment/individual. The frequencies of dicentrics, acentrics (excess) and aberrant metaphases were recorded. For statistical comparison of a particular aberration type between individuals or groups the 'Z-test' (test of equality of two proportions) was performed.

## RESULTS

Individuals of a particular group did not differ significantly for a specific aberration type. The data were therefore pooled for comparisons. In spontaneous yield (0-dose or untreated controls) chromosomal aberrations are mostly fragments. This when compared between the two groups of individuals did not differ significantly. In G0 stage, the Downs compared to normal controls showed a significant increase for the yield of dicentrics (1.5x) and aberrant cells (1.4x) but not for acentrics (Figure 1a). In G1, the Downs showed a significant increase for aberrant cells (1.6x), dicentrics (1.4x) and acentrics (1.8x), as illustrated in Figure 1b. When the yield of aberrations were compared between the two cell cycle stages for a particular group, it was found that in G1, the normal controls showed a significant increase for dicentrics (1.5x) and aberrant metaphases (1.2x) but not for acentrics. In contrast, the G1 compared to G0 cells of Downs showed a significant increase for dicentrics (1.4x), aberrant cells (1.4x) and acentrics (1.6x).

Post-irradiation incubation with 3AB showed no significant effect for the yield of any type of aberrations in the G0 stage in either group. The effect was profound for all types of aberrations in G1 stage of both the groups (Figure 1a and 1b). For normal controls, almost similar-fold of increase for dicentrics and acentrics was observed (compared to only X-ray treatment) whereas in Downs the yield of dicentrics was slightly

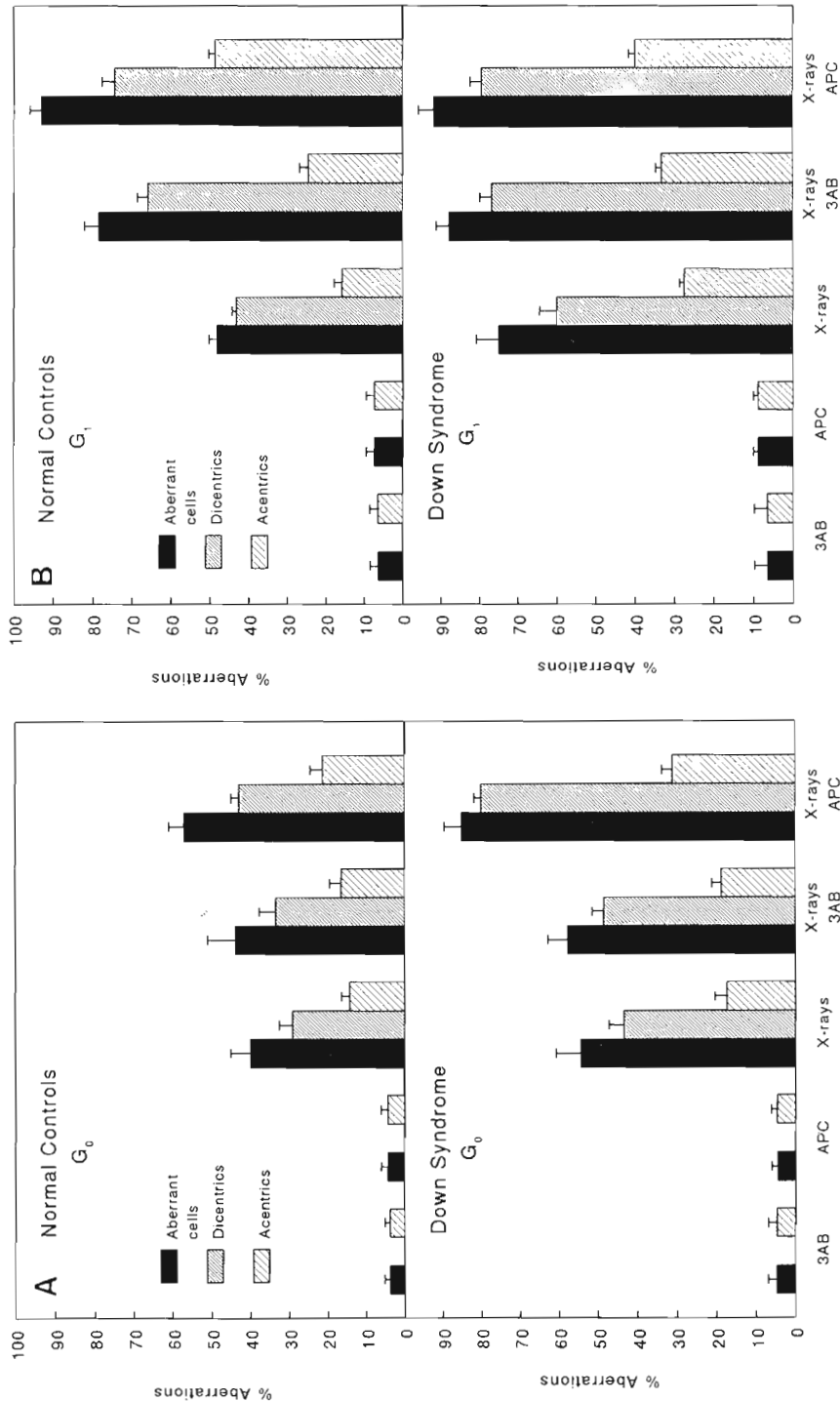


Figure 1 - The effects of 3AB or APC incubation (2 h) on the frequency of chromosome aberrations induced by X-rays (2 Gy) in unstimulated ( $G_0$ ) lymphocytes (A) and stimulated ( $G_1$ ) lymphocytes (B) of normal control and Down syndrome individuals.

higher than acentrics (Figure 1b). The overall potentiating effect of 3AB was greater in normal controls than in Downs (Figure 1a and 1b).

Post-irradiation incubation with APC in contrast to 3AB showed a significant potentiating effect for all types of aberrations in both the groups and the various stages of the cell cycle. In unstimulated lymphocytes (G<sub>0</sub> stage) the potentiating effect of APC showed an almost similar-fold increase for dicentrics and acentrics in both groups (Figure 1a). It showed higher yield of all types of aberrations in unstimulated lymphocytes of Downs when compared to normal controls. In stimulated condition (G<sub>1</sub> stage), although the yield was higher for both dicentrics and acentrics, the latter showed a much greater increase compared to the former, especially in normal controls. When compared with the X-ray treatment given in the stimulated condition, the APC showed less potentiating effects in Downs compared to normal controls (Figure 1b). The yield of all types of aberrations after APC treatment increased in normal controls compared to Downs after stimulation. Both the repair inhibitors were not found to be clastogenic when treated alone in either of the cell cycle stages studied.

## DISCUSSION

Increased yield of dicentrics and aberrant metaphases, and not of acentrics following X-ray irradiation in PHA-stimulated compared to unstimulated lymphocytes of normal individuals observed in the present study is consistent with earlier reports (Holmberg and Jonasson, 1974; Beek and Obe, 1977; Gundy and Bender, 1984), though the increase in the yield of dicentrics as well as acentrics found by Leonard and Mertz (1983) and no difference in the yield of either of the aberrations found by Carrano (1975) and Shafik *et al.* (1988), do not conform to our observations. The reasons for increased chromosomal radiosensitivity in stimulated compared to unstimulated lymphocytes are not known, though. Compaction of chromatin has been shown to modify many radiobiological end points including DNA damage induction (Warters and Childers, 1982; Oleinick *et al.*, 1984), DNA damage and repair (Wheeler and Wierowski, 1983) and cell clonogenicity (Dewey *et al.*, 1972). Each of these end points are closely related with the induction of chromosomal aberrations.

The observed potentiating effect of 3AB is in accordance with studies showing increased yield of aberrations only in stimulated lymphocytes (Pantelias *et al.*, 1986; Wiencke and Morgan, 1987). This indicates a possible influence of poly (ADP-ribose) on X-ray-induced genetic damage in stimulated or transcriptionally active phase of the lymphocytes. In human lymphocytes, poly (ADP-ribose) polymerase activity increases after mitogen stimulation and fluctuates as the cells progress through the cell cycle

(Berger *et al.*, 1982; Johnstone and Williams, 1982; Carson *et al.*, 1986). It is therefore possible that the inhibition of ADP-ribosylation after radiation-induced damage could differ in stimulated and unstimulated lymphocytes. This may account for the difference in the potentiating effects of 3AB in our study.

In case of Downs, the potentiating effect was less than for normal controls. This implies that either 3AB has a limited effect on stimulated Down lymphocytes, possibly due to changes in poly (ADP-ribose) polymerase level or the level of aberrations are undetectable due to cell cycle delay or cell death caused by extensive genetic damage. Such a saturation level of aberration after 3AB treatment has also been found in two X-ray-sensitive cell lines, xrs 5 and xrs 6 derived from Chinese hamster ovary (CHO-K1) cells (Darroudi and Natarajan, 1987). MacLaren *et al.* (1989) have reported an increased frequency of chromosomal aberrations in unstimulated lymphocytes from Downs as a result of treatment with X-rays in presence of 3AB. They have suggested that lymphocytes from Downs are more sensitive to the inhibition of poly (ADP-ribose) polymerase than the normal lymphocytes. It is difficult to provide an explanation for these contrasting results. Increased aberrations in stimulated lymphocytes of normal controls compared to Downs at least indicates that they behave differently following incubation with poly (ADP-ribose) polymerase inhibitor.

Increased levels of dicentrics and acentrics with APC post-treatment in both unstimulated and stimulated conditions were also found by Bender and Preston (1982), Natarajan *et al.* (1982) and Moore and Bender (1987). There was, however, no clastogenic effect of APC as reported in some studies (Bender and Preston, 1982; Bender and Moore, 1988). In stimulated lymphocytes of Downs, like 3AB, the potentiating effect of APC was less effective than in normal controls. The stage specific effects of APC (Moore and Bender, 1987) or enzymes associated with the production of chromosome aberrations might have some role. Increased DNA polymerase activity in PHA-stimulated lymphocytes of Downs has been reported to be higher than in normal controls (Agarwal *et al.*, 1970).

In conclusion, lymphocytes from Downs, when compared to normal controls, behave differently in the processing of X-ray-induced genetic damage, both in the presence or absence of 3AB and APC. This appears to be an inherent characteristic as histophysiological abnormalities of the thymus gland in these patients lead to qualitative changes in the thymic dependent cells (Levin, 1987).

## ACKNOWLEDGMENTS

This work was supported by DAE (Block Assistance), Bombay and UGC (Special Assistance), New Delhi. We are grateful to Professor Dr. A.T. Natarajan (University of Leiden, The Netherlands) for going

through the manuscript. We would like to thank the staff of Pediatrics Department (Banaras Hindu University) for their help in procuring the blood samples.

## RESUMO

Amphidicolin (APC) e 3-aminobenzamida (3AB) foram utilizados para elucidar o papel de processos de reparo de DNA no aumento de aberrações induzidas por Raio-X em culturas de linfócitos de pacientes com Síndrome de Down, comparadas com controles normais. Estimulados por Fito-hemaglutinina (PHA) (Fase G1), os controles normais mostraram um aumento significativo na produção de dicêntricos, mas não de acêntricos. Em culturas de Downs as células estimuladas mostraram um aumento significativo na produção de dicêntricos e acêntricos. O efeito de APC, em contraste, foi menos pronunciado sem estimulação. Na condição estimulado, como para 3AB, o efeito de APC foi maior em controles normais. O processamento diferencial de dados genéticos induzidos por Raio-X em linfócitos periferais de Downs, é sensível a presença de APL e 3AB. É provável que ambos, DNA polimerase  $\alpha$  e poly (ADP-ribose) polimerase tem comportamento diferenciado intrínseco nos linfócitos de Downs sensíveis a PHA.

## REFERENCES

- Agarwal, S.S., Blumberg, B.S., Gerstley, B.J.S., London, W.T., Sutnick, A.I. and Loeb, L.A. (1970). DNA polymerase activity as an index of lymphocyte simulation: studies in Down's syndrome. *J. Clin. Invest.* 49: 161-169.
- Beck, B. and Obe, G. (1977). Differential chromosomal radiosensitivity within the first G1-phase of the cell cycle of early-dividing human leukocytes *in vitro* after stimulation with PHA. *Human Genet.* 35: 209-218.
- Bender, M.A. and Moore, R.C. (1988). Dose relationships for different effects of aphidicolin in human blood leukocytes. *Mutation Res.* 198: 227-231.
- Bender, M.A. and Preston, R.J. (1982). Role of base damage in aberration formation: Interaction of Aphidicolin and X-rays. In: *DNA Repair, Chromosome Alterations and Chromatin Structure* (Natarajan, A.T., Obe, G. and Altman, H., eds.). Elsevier, Amsterdam, pp. 37-46.
- Benjamin, R.C. and Gill, D.M. (1980). Poly (ADP-ribose) synthesis *in vitro* programmed by damaged DNA. A comparison of DNA molecules containing different types of strand breaks. *J. Biol. Chemistry* 255: 10502-10508.
- Berger, N.A., Sikorski, G.W., Potzold, S.J. and Kurohara, K.K. (1979). Association of poly (adenosine diphosphoribose) synthesis with DNA damage and repair in normal human lymphocytes. *J. Clin. Invest.* 63: 1164-1171.
- Berger, N.A., Berger, S.J., Sikorski, G.W. and Catino, D.M. (1982). Amplification of pyridine nucleotide pools in mitogen stimulated human lymphocytes. *Exp. Cell Res.* 137: 79-88.
- Carrano, A.V. (1975). Induction of chromosomal aberrations in human lymphocytes by X-rays and fission neutrons: dependence on cell cycle stages. *Radiat. Res.* 63: 403-421.

- Carson, D.A., Seto, S., Wasson, D.B. and Carrera, C.J. (1986). DNA-strand breaks, NAD metabolism and programmed cell death. *Exp. Cell Res.* 164: 273-281.
- Countryman, P.I., Heddle, J.A. and Crawford, E. (1977). The repair of X-ray-induced chromosomal damage in trisomy 21 and normal, diploid lymphocytes. *Cancer Res.* 37: 52-58.
- Darroudi, F. and Natarajan, A.T. (1987). Cytological characterization of Chinese hamster ovary X-ray-sensitive mutant cells xrs 5 and xrs 6. I. Induction of chromosomal aberrations by X-irradiation and its modulation with 3-aminobenzamide and caffeine. *Mutat. Res.* 177: 133-148.
- Dewey, W.C., Noel, J.S. and Dettor, C.M. (1972). Changes in radiosensitivity and dispersion of chromatin during the cell cycle of synchronous Chinese hamster cells. *Radiat. Res.* 52: 373-394.
- Durkacz, B.W., Omidiji, O., Gray, D.A. and Shall, S. (1980). (ADP-ribose)<sub>n</sub> participates in DNA excision repair. *Nature* 283: 593-596.
- Goto, K., Akematsu, T., Shimazu, H. and Sugiyama, T. (1975). Simple differential Giemsa staining of sister chromatids after treatment with photosensitive dyes and exposure to light and the mechanism of staining. *Chromosoma* 53: 223-230.
- Gundy, S. and Bender, M.A. (1984). Increased yield of exchanges but not of deletions in X-irradiated human peripheral lymphocytes following phytohaemagglutinin stimulation. *Radiat. Res.* 97: 519-525.
- Holmberg, M. and Jonasson, J. (1974). Synergistic effect of X-ray and UV irradiation on the frequency of chromosome breakage in human lymphocytes. *Mutat. Res.* 23: 213-221.
- Huberman, J.A. (1981). New views of the biochemistry of eucaryotic DNA replication revealed by aphidicolin, an unusual inhibitor of DNA polymerase  $\alpha$ . *Cell* 23: 647-648.
- Ikegami, S., Taguchi, T., Ohashi, M., Oguro, M., Nagano, H. and Mano, Y. (1978). Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase  $\alpha$ . *Nature* 275: 458-460.
- Jha, A.N. and Sharma, T. (1991). Enhanced frequency of chromosome aberrations in workers occupationally exposed to diagnostic X-rays. *Mutat. Res.* 260: 343-348.
- Johnstone, A.P. and Williams, G.T. (1982). Role of DNA breaks and ADP-ribosyl transferase activity in eukaryotic differentiation demonstrated in human lymphocytes. *Nature* 300: 368-370.
- Leonard, J.C. and Mertz, T. (1983). The influence of cell cycle kinetics on the radiosensitivity of Down's syndrome lymphocytes. *Mutat. Res.* 109: 111-121.
- Levin, S. (1987). The immune system and susceptibility to infections in Down's syndrome. In: *Oncology and Immunology of Down Syndrome* (McCoy, E.E. and Epstein, C.J., eds.). Alan R. Liss, New York, pp. 143-162.
- MacLaren, R.A., Au, W.W. and Legator, M.S. (1989). The effect of 3-aminobenzamide on X-ray induction of chromosome aberrations in Down syndrome lymphocytes. *Mutat. Res.* 222: 1-7.
- Moore, R.C. and Bender, M.A. (1987). The synergistic effect of aphidicolin on the yield of X-ray-induced chromosome aberrations throughout the cell cycle in JU 56 cells. *Radiat. Res.* 110: 385-395.
- Morimoto, K., Kaneko, T., Iijima, J. and Koizumi, A. (1984). Proliferation kinetics and chromosome damage in trisomy 21 lymphocyte cultures exposed to X-ray and bleomycin. *Cancer Res.* 44: 1499-1504.
- Natarajan, A.T., Csukas, I., Degrassi, F., van Zeeland, A.A., Palitti, F., Tanzarella, C., de Salvia, R. and Fiore, M. (1982). Influence of inhibition of repair enzymes on the induction of chromosomal aberrations by

- physical and chemical agents. In: *DNA Repair, Chromosome Alterations and Chromatin Structure* (Natarajan, A.T., Obe, G. and Altman, H., eds.). Elsevier, Amsterdam, pp. 47-59.
- Oleinick, N.L., Chiu, S.M. and Friedman, L.R. (1984). Gamma radiation as a probe of chromatin structure: damage to and repair of active chromatin in metaphase chromosome. *Radiat. Res.* 98: 629-641.
- Pantelias, G.E., Politis, G., Sabani, C.D., Wiencke, J.K. and Morgan, W.F. (1986). 3-Aminobenzamide does not affect X-ray-induced cytogenetic damage in G0 human lymphocytes. *Mutat. Res.* 174: 121-124.
- Preston, R.J. (1981). X-ray-induced chromosome aberrations in Down lymphocytes: an explanation of their increased radiosensitivity. *Environ. Mutagen.* 3: 85-89.
- Purnell, M.R. and Whish, W.J.D. (1980). Novel inhibitors of poly (ADP-ribose) synthetase. *Biochem. J.* 185: 775-777.
- Sasaki, M.S., Tomomura, A. and Matsubara, S. (1970). Chromosome constitution and its bearing on the chromosomal radiosensitivity in man. *Mutat. Res.* 10: 617-633.
- Shafik, H.M., Au, W.W. and Legator, M.S. (1988). Chromosomal radiosensitivity of Down syndrome lymphocytes at different stages of the cell cycle. *Human Genet.* 78: 71-75.
- Warters, R.L. and Childers, T.T. (1982). Radiation-induced thymine base damage in replicating chromatin. *Radiat. Res.* 90: 564-574.
- Wheeler, K. and Wierowski, J. (1983). DNA accessibility: A determinant of mammalian cells differentiation? *Radiat. Res.* 93: 312-318.
- Wiencke, J.K. and Morgan, W.F. (1987). Cell cycle-dependent potentiation of X-ray-induced chromosomal aberrations by 3-aminobenzamide. *Biochem. Biophys. Res. Commun.* 143: 372-376.

(Received July 24, 1992)