J. Egypt. Natl. Cancer Inst. 1 : 65-75, 1982.

Changes in Nuclear Size and DNA Content of Carcinoma of Cervix After Fractionated Irradiation

NADIA MOKHTAR, Ph. D. ; IBRAHIM EZZAT, M. D.;
MOHAMED HUSSEIN, Ph. D. and NABIL
EL-BOLKAINY, Ph. D.

Departments of Pathology and Radiotherapy, National Cancer Institute and Department of Hygiene and Public Health, Faculty of Medicine, Cairo University.

Abstract

The effect of conventional fractionated irradiation (5 Fr of 2.00 Gy/week) on nuclear size and DNA content of squamous cell carcinoma of the cervix was evaluated in 6 patients. Three consecutive biopsies were taken from each patients ; prior to irradiation, after 20.00 Gy and after 40.00 Gy. The nuclear diameter was measured using eyepiece micrometer. Cytophotometlic DNA analysis was made on Feulgen-stained sections. A total of 1800 tumor nuclei and 1200 normal epithelium nuclei were examined ; 100 nuclei per sample. Progressive increase in the nuclear size was observed in tumor cells with increase of the dose. In normal cells, however, this increase was only observed after the high dose. Prior to irradiation, the distribution of DNA content in tumor nuclei showed a primary mode between the diploid and tetraploid range. After irradiation, however an irregular DNA distribution was observed with disappearance of primary mode, a wide scatter over the extreme values and a relative increase of the mean value. Normal nuclei showed only a slight increase of DNA mode after irradiation. The radiobiologic explanations of these changes are presented together with their possible clinical implications particulary in predicting tumor responsiveness to radiotherapy.

Introduction

QUANTITATIVE optical cytochemistry and cytometry represent an objective approach to determine many different parameters of tumor cells. Cytophotometric analysis of DNA content (Caspersson, 1979) and measurement of nuclear size (Fossa and Kaallhus 1976 ; Bogaert and Muylder 1980) are among the most important parameters studied . These investigations have contributed considerably to the development of our

present knowledge on normal and neoplastic growth. Thus, malignant cells often exhibit increased amounts of DNA as well as increased nuclear size and these changes may have prognostic implications.

The possibility also exists that nuclear size and DNA concentration may represent cellular features which influence the radiosensitivity of a tumor cell population (Sparrow et al, 1963). Previous studies in this field are generally scanty and only involved experimental animals (Caspersson et al 1958 ; Conger & Linton 1973). The present study was undertaken to characterize the changes in nuclear size and DNA content in carcinoma of the uterine cervix treated by irradiation. The long-term objective is to determine whether such changes could be a valid indicator of the irradiation responsiveness of the tumors.

Materials and Methods

Six patients with carcinoma of uterine cervix were included in this study, all were treated at the Radiotherapy Department of the National Cancer Institute during the years 1980 and 1981. The tumors were staged according to the FIGO classification and typed histologically following the WHO system. A minimum follow up period of at least 6 months was accomplished for all cases. The clinicopathological data of this series is presented in Table 1. All the patients presented with a relatively advanced tumors, and hence radiotherapy was the sole line of treatment.

Irradiation was carried out using a telecobalt unit operating at 80 SSD with a dose rate of about 1.2 Gy per minute. The whole pelvis was irradiated using 2 paraliel opposing portals, 15 x 15 cm each. Tumor dose was calculated at the mid-pelvis, and the two fields were treated every day. A total dose of 40.00 Gy was delivered at the rate of 2.00 Gy/day over a period of 4 weeks, five fractions every week.

Table 1. Clinicopathological Data

Case No	Age	Stage	Hist. Type *	Follow up/6m
1	55	II B	Large cell	Free
2	38	III A	Large cell	Free
3	54	III A	Small cell	Residual
4	62	III B	Small cell	Residual
5	58	III A	Small cell	Free
6	40	III A	Small cel	Free

* All cases were non-keratinising squamous cell carcinoma classified according to WHO histological typing (1979).

Three biopsies were taken from each patient, one before irradiation, another after delivering 20.00 Gy, and a third after completing the 40.00 Gy. Biopsy samples included both tumor tissue as well as adjacent normal ectocervical epithelium which served as an internal control. From each paraffin block, 3 tissue sections were cut 5 microns thick. One was stained with Hematoxylin and Eoosin, and the others with the Feulgen reaction for DNA (Stowell 1945), using a hydrolysis time of 60 minutes in 5 N HCL at room temperature.

The DNA measurements were carried out using a Leitz MPV compact microscope photometer (E. Leitz company, West Germany) fitted with a spectrum filter of 546 nm transmission maximum. All DNA determinations were made according to the «plug» technique of Swift and Rasch (1956). For each tissue section, the nuclear diameters (in microns) and light transmission values (T) of 100 nuclei were recorded. For non-round nuclei, the mean of the longest and shortest diameters was determined. The DNA content of nuclei was expressed in arbitrary units (A.U.) where A.U. = Nuclear extinction x nuclear area. The extinction (E) was determined from equation : $E = \log (1 \div \frac{T}{100})$ where T represents transmission. The nuclear area (NA) was obtained from the equation : $NA = 3.14 \times r^2$, where r is the radius of the nucleus. Data analysis was made using a minicomputer HP 97 (Hewlett-Packard, U.S.A.) and the results were expressed as frequency distributions of nuclear diameters and DNA arbitrary units.

Results

Nuclear Size :

The effect of irradiation on the nuclear diameter of the normal squamous epithelium of the uterine cervix is presented in Fig. 1. Significant enlargment of the nuclei was only documented after attaining the full dose 40.00 Gy. The mean nuclear diameter before irradiation was 11.1 microns, 10.7 microns after delivering 20.00 Gy, and reached 13.9 microns after 40.00 Gy.

The increase of nuclear size of cancer cervix after irradiation is evident in Table 2. and Fig. 2. Prior to irradiation, the peak of nuclear size was between 5 and 10 microns in two tumors (case 3 & 4), whereas in all the other tumors the peak was between 10 and 15 microns. Following irradiation, enlargment of nuclei was observed at both doses 20.00 and 40.00 Gy with a peak shift to higher values with the increase of the dose (Fig. 2). After irradiation the scatter of nuclear size covered a wider range reflecting variability in nuclear diameter. This phenomenon of nuclear pleomorphism induced by irradiation was also remarkable by histopathologic study (Fig. 3).

DNA Content :

The DNA distribution of the normal non-irradiated cervical epithelium showed a narrow mode at 40 arbitrary units (Fig. 4) representing the normal diploid (2C) of the epithelial population. After irradiation, an increase of DNA content was observed with the increase of the dose. Thus, at 20.00 Gy the mode of DNA distribution shifted very close to the tatraploid value (4C), and at 40.00 Gy, the mode slightly exceeded the tetraploid value (Fig. 4).

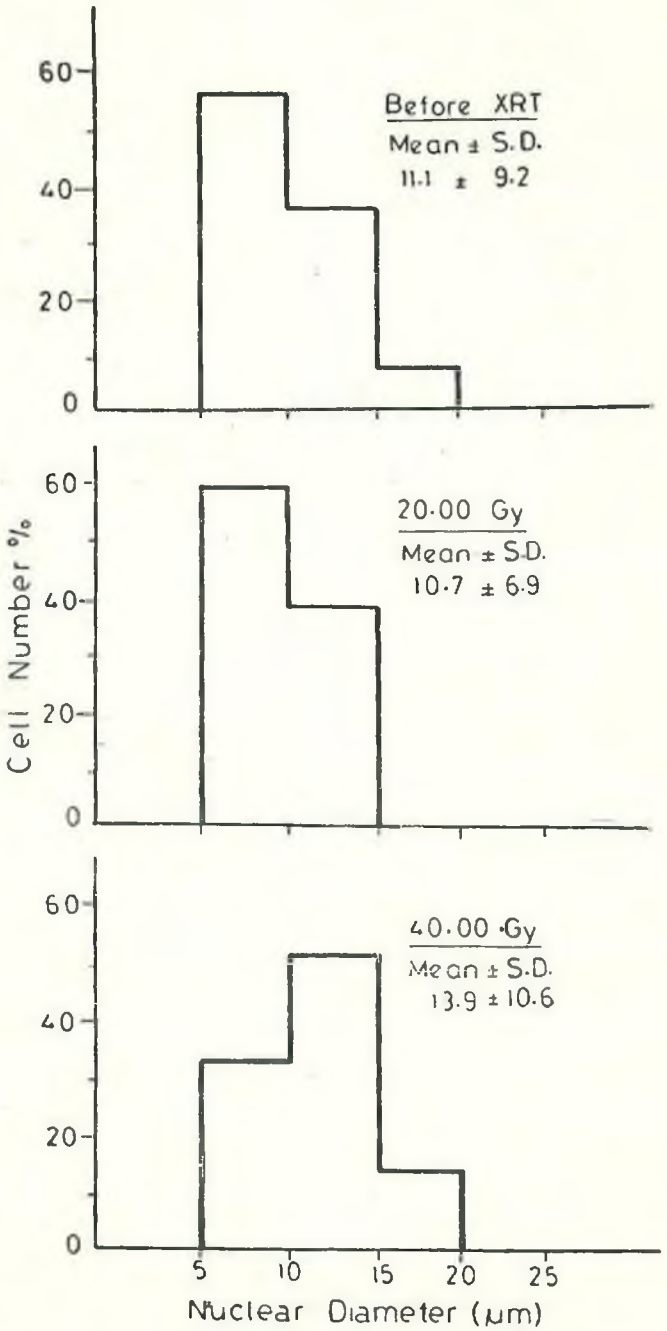

Fig. 1. The effect of irradiation on nuclear diameter of the normal squamous epithelium of human uterine cervix.

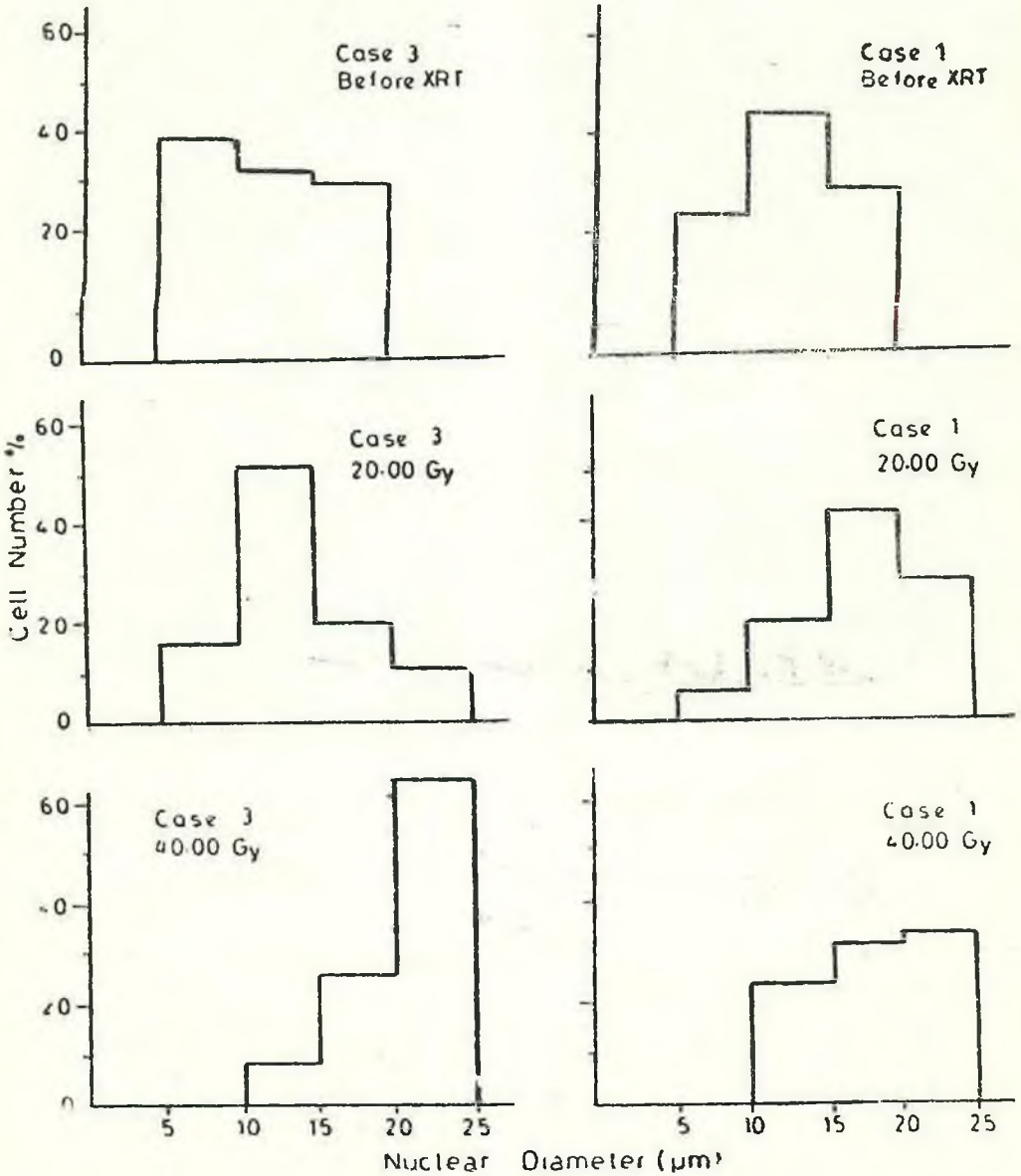

Fig. 2. The effect of irradiation on the nuclear diameter of squamous carcinoma of the cervix.

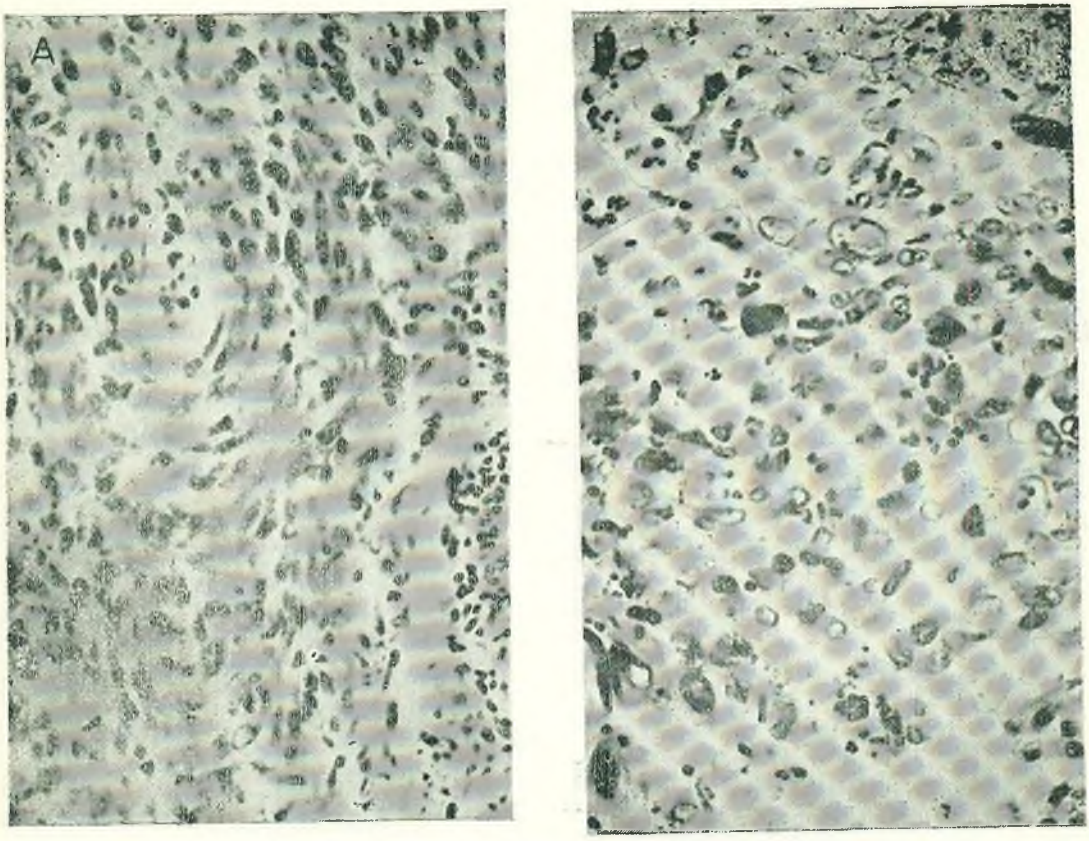

Fig. 3. Histopathologic changes after irradiation of carcinoma of the cervix (case 3), H & E stain x 400. A. Non-keratinizing grade 3 carcinoma before irradiation.

B. The same tumor after irradiation showing variation of nuclear size and density.

Table 2. Effect of Irradiation on the Nuclear Diameter of Carcinoma of the Cervix.

Case	Preirradiated	20 GY	40 GY
1	13.7±12.9*	17.4±14.3	19.8±13.9
2	14.6±13·7	14.6±9.1	18.6±12.7
3	12.3±11.1	14.9±12.2	21.8±10.6
4	10.1±10.9	15.6±13.1	17.9±12·3
5	12.2±9.9	16.5±13.6	15.8±12.1
6	12.3±10.1	17.3±6.8	18.1±11.7
Total	12.5±1.5	16.1±1.2	18.7±2.0

* Mean ± standard deviation of 100 nuclei measured in micrometers.

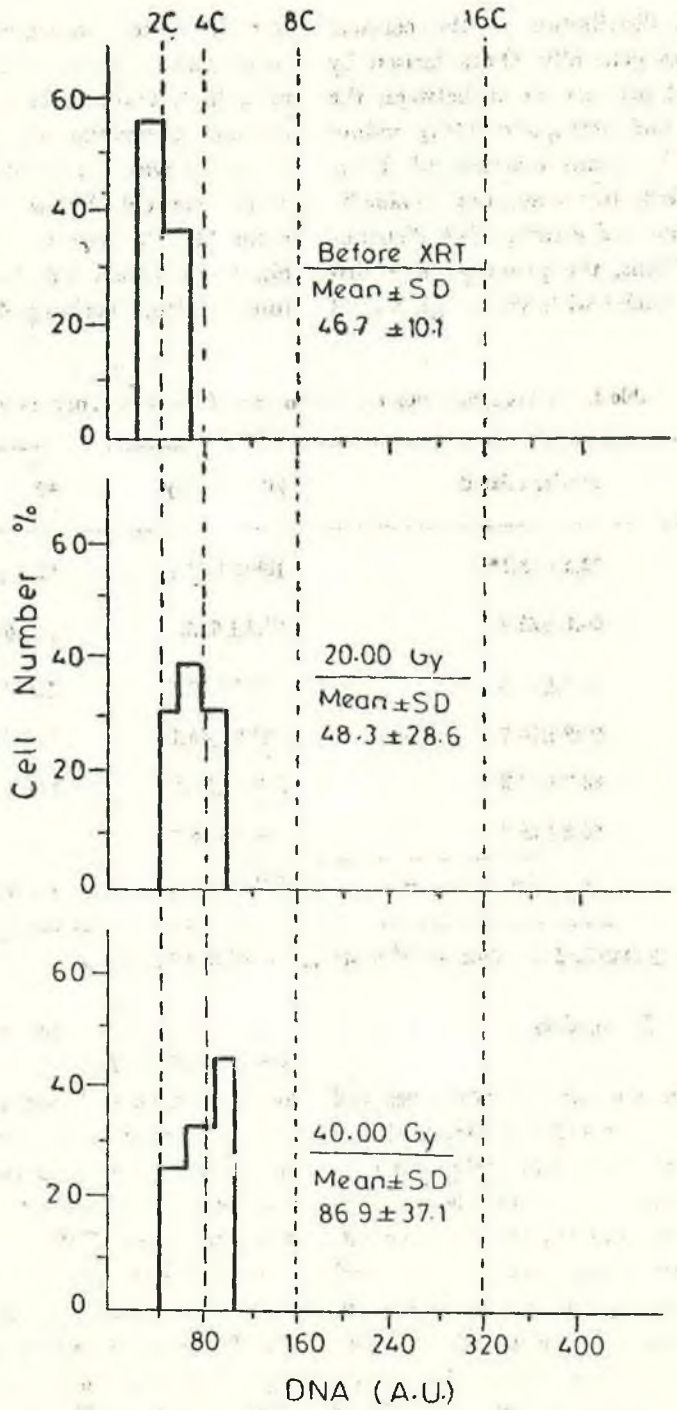

Fig. 4. The effect of irradiation on the DNA content of normal cervical epithelium.

The DNA distribution of the cervical carcinomas was generally characterised by the presence of primary mode between the diploid (2C) and tetraploid (4C) values (Fig. 5). A significant increase of DNA values was evident after irradiation (Table 3) with a very wide and erratic DNA distribution (Fig. 5). Thus, the primary mode disappeared and nuclei with very high values of DNA content were observed (8C and 16C) in all cases. Such changes were prominant at a high doose level of 40.00 Gy. An additional observation noted in two cases (Case 4 and 6) was the finding in some nuclei of DNA content below the normal diploid value (Fig. 5, Case 6). This rare hypodiploid phenomenon was only observed in two tumors after receiving 40.00 Gy.

Table 3. Effct of Irradiation on dna Content of Carcinoma of the Cervix.

Case	Pre-irradiated	20 Gy	40 Gy
1	72.5±18.1*	108·1±68.8	92.7±51.9
2	64.8±23.7	69.3±60.2	132.6±71.1
3	78·8±27.5	137.1±53.6	129.7±49.7
4	69·9±14.7	163.9±44.1	106·6±81.5
5	86.7±37.2	115.8±38.5	186.1±97.7
6	60.8±29.7	144.7±36.7	122.9±66.5
Mean	72.3±9.4	123.1±33.2	128.4±32.0

* Mean ± standard deviation of 100 nuclei measured in arbitrary unirs.

Discussion

Increase in nuclear size was observed after irradiation of uterine cervix, and this change was more marked in malignant cells than normal cells. Three possible mechanisms could explain this nuclear enlargement, namely : raised DNA content, increased nuclear function and nuclear degeneration. Increase in nuclear size as a result of increase in DNA content may be the result of polyploidy (Hobik and Grundmann 1962) or increased proliferative activity (Andersson and Agrell 1972). Increased nuclear size and decreased chromatin concentration may be the morphologic expression of the despiralizing of chromatin, a process which usually is connected with increased nuclear function (Brown 1966). Finally, increase in nuclear size may be the result of nuclear degeneration and is mainly due to water imbalance with water intake as well as degeneration of nucleic acids and nucleoproteins (Frost, 1969).

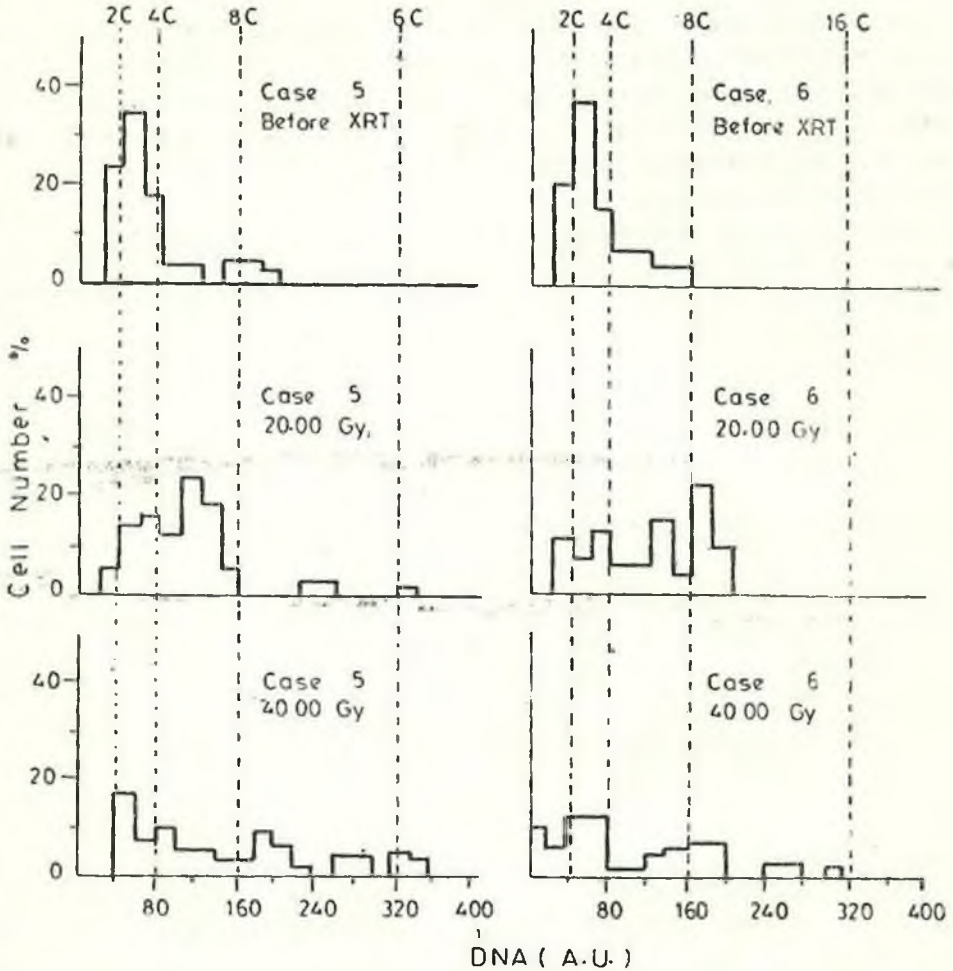

Fig. 5. The effect of irradiation on the DNA content of carcinoma of the cervix.

Quantitative cytophotometric DNA analysis of the nuclei of tumor cells supplies valuable information, superior to chromosomal analysis, since large numbers of cells at all stages of the cell cycle may be measured by this method within a reasonable time. In the present investigation, the DNA distribution of carcinoma of the cervix prior to irradiation is essentially similar to that repoorted by other investigators (Atkin & Richards, 1956). A welldefined primary mode was evident between the diploid and tetraploid values. This restricted modal distribution of DNA values is explained by the stem-line phenomenon which has been described in other tumors (Sandritter 1965). Under the stem-line concept, although an extremely heteroploid population complete

DNA synthesis, only a portion of this population successfully completes cell division and produces daughter cells. This proliferating population is generally composed of one, or a limited number of cell-lines, of rather homogeneous chromosomal content and is referred to as the stem line (S) of the neoplasm. The concept implies that a large proportion of neoplastic cell population, usually containing the most bizzare cell types, does not contribute significantly to the growth of the tumor.

The main effect of irradiation on the DNA of tumors were the disappearance of the primary mode and erratic distribution of DNA with a broad scatter over a wide range. These findings are in agreement with the previous reports (Richards and Atkin 1959 ; Sugimori & Gusberg 1969). Disappearance of the primary mode could be explained by the inhibitory effect of irradiation on the stem-line which represents the growth fraction of the tumor cell population.

Another important observation after irradiation was the increase of DNA content of the nuclei. This is explained by several possible mechanisms. Caspersson and associates (1958) suggested that the primary effect of irradiation is not to upset the sequence of chemical reactions leading to DNA synthesis, but rather involves some stage of the mitotic process. Accordingly, polyploid cells appear as a result of endomitosis and arrest of cell division. Another explanation is the presence of aneuploidy in many of the tumor cells. Thus, the marked variability of DNA content is mainly the result of abnormal mitosis in the tumor cell population with marked chromosomal aberrations. Finally, another possible mechanisms of increased DNA after irradiation is the production of double-breaks in DNA helix during the process of replication followed by postirradiation or recombination repair (Fujiwara and Kondo, 1974).

In two irradiated tumors, DNA content below the normal deploid value was observed in some nuclei. These hypodiploid nuclei are probably the outcome of abnormal mitosis with unequal distribution of chromosomes between the daughter cells. It is probable that cells with extreme DNA values observed in the present investigation are sterile non-proliferating cells.

An increase in nuclear size and a wide scatter of DNA distribution were the main changes observed in the irradiated tumors. These findings may have important practical implications in the future. Since the tumor type selected for this study is rather a radioresponsive tumor, these parameters may be considered as good indicators of radiation responsiveness. Accordingly, this model offers a sensitive method which could be used to compare the effectiveness of different fractionated radiotherapeutic schedules for a given tumor. Another value of this method is its potential use in the future to predict the radiation response of tumors. An additional advantage of this cytophotometric technique is its applicablity on cytologic samples from the uterine cervix, hence allowing early detection of recurrence of the disease after irradiation (Okagaki et al, 1974). It was not possible in the present study to correlate the extent of nuclear changes with the clinical end results because of the small number

of cases. Further studies are currently being done using this method in experimental animal rumors as well as different human tumors of variable radioresponsiveness.

References

1. ANDERSSON, G. K. A. and AGRELL, I. P. S. (1972) : Cytoplasmic and nuclear growth during the proliferation of Ehrlich ascites tumor cells in mice. Virchows Arch. Abt. B. Zellpath. 11 : 1-10.
2. ATKINS, N.B. and RICHARDS, B.M. (1956) : Deoxyribonucleic acid in human tumours as measured by microspectrophotometry of Feulgen stain : a comparison of tumours arising at different sites. Brit. J. Cancer, 10 : 769-786.
3. BOGAERT, L. and MUYLDER, C. (1980) : Nuclear diameters of breast cancer cells in tissue sections. Analyt. Quantit. Cytol. 2 : 55-58.
4. BROWN, S.W. (1966) : Heterochromatin. Science, 151 : 417-425.
5. CASPERSSON, T. ; KLEIN, E. and RINGERTZ, N.R. (1958) : Cytochemical studies on some effects of X-irradiation on three ascites tumors ; Cancer Res. 18 : 857-862.
6. CASPERSSON, T.O. (1979) : Quantitative tumor cytochemistry. Cancer Res. 33 : 2341-2355.
7. CONGER, A.D. and LINTION, J.H. (1973) : Nuclear volumes, DNA content and radiosensitivity in whole-body irradiated amphibians. Radiat. Res. 54 : 69-101.
8. FOSSA, S.D. and KAALHUS, O. (1976) : Nuclear size and chromatin concentration in transitional cell carcinoma of the human urinary bladder. Beitr. Path. Bd. 157 : 109-125.
9. FROST, J.K. (1969) : The cell in health and disease, Williams and Wilkins Co., Baltimore, p. 36.
10. FUJIWARA, Y. and KONDO, T. (1974) : Post replication repair of ultravoilet damage to DNA in Xeroderma pigmentosum, other human and mouse cells in culture, J. Rad. Res. 15 : 18.
11. HOBIK, H.P. and GRUNDMANN, E. (1962) : Quantitative veranderungen der DNA und der RNS in der Rattenleberzelle wahrend der carcinogenese durch Diathynitrosamin, Beitr. Path. Anat. 127 : 25-48.
12. OKAGAKI, T., MEYER, A. A. and SCIARRA, J.J. (1974) : Prognosis of irradiated carcinoma of cervix uteri and nuclear DNA in cytologic postirradiation dysplasia. Cancer, 33 : 647-652.
13. RICHARDS, B.M. and ATKINS, N.B. (1959) : DNA content of human tumors - changes in uterine tumors during radiotherapy and their response to treatment. Brit. J. Cancer, 13 : 788-800.
14. SANDRITTER, W. (1965) : DNA content of tumors cytophotometric measurements. Europ. J. Cancer, 1 : 303-307.
15. SPARROW, A.H. ; SCHAIRER, L.A. and SPARROW, R.C. (1963) : Relationship between nuclear volumes, chromosome number and relative radiosensitivities. Science, 141 : 163-166.
16. STOWELL, R. (1945) : Feulgen reaction for thymonucleic acids. Stain Technol., 20 : 45-58.
17. SUGIMORI, H. and GUSBERG, S. B. (1969) : Quantitative measurement of DNA content of cervical cancer cells before and after test dose radiation. Am. J. Obstet. Gynecol., 104 : 829-838.
18. SWIFT, H. and RASCH, E. (1956) : Microscoectrophotometry with visible light. In, Physical Technique in Biological Research-Cell and tissue, vol. 3. New York, Academic Press, pp. 354-400.