EXFOLIATIVE CYTOLOGY IN THE PROGNOSIS OF MALIGNANT DISEASE OF THE UTERINE CERVIX TREATED BY IONIZING RADIATION

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The present position of exfoliative cytology in the prognosis of malignant disease of the uterine cervix treated by ionizing radiation is not clear.

RADIATION CHANGES OCCURRING IN BENIGN CELLS

The appearance of cellular changes subsequent to irradiation is a well-known phenomenon and the changes have been fully described. Vacuolation of the cytoplasm, increased cell size, increased nuclear size and multinucleation are changes which have been recorded in benign cells. Parabasal and basal cells often show considerable vacuolation, which led Graham and Graham[1] to suggest that these changes could have a prognostic value. They postulated that the degree of change would be indicative of the sensitivity of the tumour to irradiation, 'RR' or radiation response being the percentage of cells showing radiation changes. Routine staining by the Papanicolaou method, using Harris' haematoxylin, often shows the nucleus as an even, glassy, slate-blue structure.

Vacuolation of the cytoplasm is perhaps too often ignored as a degenerative change and attention focussed solely on nuclear changes. Vacuolation is a manifestation of a disordered metabolism following upon irradiation, possibly the visible result of faulty nucleic acid synthesis.[2] It is possibly, in part, caused by RNA transfer from cytoplasm to nucleus. Gusberg,[3] using specific nuclear staining to evaluate cytochemical changes, suggested that a good response to irradiation was shown if the trend was towards a higher percentage of B cells (relatively rich in RNA) with a decrease in A cells (relatively rich in DNA) and cells in mitosis. Radiation has been shown to inhibit DNA synthesis while having little effect on RNA activity. Atkin[4] has reported a relationship between nuclear size and prognosis following radiotherapy based on nuclear measurement and DNA estimations on Feulgen-stained sections of cervical tumours.

Increased cell size and multinucleation with juxtaposition of macro- and micronuclei within the same cell border are visible signs of chromosomal aberration. A study of irradiation changes by time lapse photography[2] has shown that fibroblasts entering mitosis 15 - 20 hours after irradiation often remained in this state for very long periods and, in fact, seldom completed the process of division. Multinucleated giant epithelial cells were often the result of such abortive mitoses.

As a result of irradiation, chromosome breakages may occur and chromosomal abnormalities develop in metaphase cells. Breakages may occur even in the more radioresistant cells such as nerve and bone cells. Usually these latter will continue to function normally. Where cells ultimately divide weeks, months or even years later the damage will become apparent. This fact partly explains one of the most puzzling aspects of post-irradiation cytology, the appearance of bizarre irradiation changes in smears taken many years after the end of treatment.

RADIATION CHANGES OCCURRING IN MALIGNANT CELLS

Malignant cells after irradiation therapy are often bizarre in the extreme, but their appearance may be more fully understood if attention is paid to the characteristics of the malignant cells, particularly in regard to the nucleus. In considering the criteria of malignant cells stress is usually placed upon hyperchromatism, but hypochromatism should not be disregarded. In uterine neoplasms it is

not unusual for cells to possess fewer than 46 chromosomes. In gastric cancer it is fairly common[5] and this accounts for one of the difficulties encountered in the interpretation of gastric smears when no obviously hyperchromatic cells are present.

Many of the cells will however possess many more than 46 chromosomes and will be seen as hyperchromatic cells. The number of chromosomes present appears to be related to the radiosensitivity of the tumour.[6] It is probable that tumours with an average of more than 60 chromosomes will be radio-resistant and those with fewer than 60 will be radio-sensitive.

In malignant cells, following upon irradiation, changes similar to those found in benign cells will occur. Mitosis is interfered with and bizarre forms appear in the smears. Multinucleated giant epithelial cells whose nuclei appear as intense, solid, slate-coloured masses, are seen together with pale, ghostly forms.

PROGNOSTIC VALUE OF POST-IRRADIATION PHENOMENA

Can these post-irradiation phenomena be utilized in tests for the prognosis of malignant disease?

This question has been debated at some length and there have been numerous proposals for their utilization. Tumour and host characteristics before treatment have been studied[7-13] as have tumour and host response during therapy.[14-21]

Tests following treatment, such as assessment of tumour status by biopsies performed at the conclusion of treatment[22] and a combination of histo-cytochemical tests[23] have also been suggested. Graham and Graham introduced the concept of SR (sensitization response) in the pre-treatment smear—i.e. host response to the tumour—and RR (radiation response)—i.e. tumour-host response to irradiation—utilizing the changes found in the basal layers as indications of response.

Many workers supported this concept while many others failed to corroborate their findings. The over-all picture tends to suggest that SR is not a reliable prognostic index as its validity is obviously affected by the endocrine state of the patient.[24] A good SR is more frequent in postmenopausal women than in those in younger age-groups. In our admittedly limited experience, RR has not proved to be a reliable indicator. A different approach was made by Wachtel,[25] who observed an association between malignant growths of the uterus and an abnormally high cornification or karyopyknotic index after the cessation of normal ovarian function in natural, surgical or post-irradiation menopause, and suggested that this could be used as a simple prognostic test.

The karyopyknotic index (KI) is defined as the percentage of squamous cells with pyknotic nuclei. It is regarded as the manifestation of the patient's oestrogen level since normally ovulating women reach peak value of cornification at ovulation and oestrogen administered to postmenopausal women raises the cornification value. KI is estimated by counting the percentage of cells with pyknotic nuclei among 200 unselected superficial and intermediate cells, although parabasal cells may, in severe inflammation, show nuclear pyknosis. Basal cells are ignored in this estimation. Observations on a small group of patients suggested that the finding of a high KI after treatment for malignant disease indicates persistence of the disease. It was postulated that tumours produce oestrogen or oestrogen-like substance and that the removal by surgery or the destruction by irradiation of viable tumour tissue would lead to a drop in the KI. Failure of the treatment would be shown by a continuing high index.

A possible complication arises from the fact that digitoxin and other cardiac glucosides display an oestrogen-like activity. It is possible that they are converted, *in vivo*, into oestrogenic compounds.[26] It is important that the full therapeutic regimen of the patient be made available to the cytologist.

THE USE OF CYTOLOGY IN THE DIAGNOSIS OF RECURRENT CARCINOMA

The appearance of malignant cells in vaginal smears, months or years after treatment, is often the earliest sign of recurrent disease and frequently precedes the appearance of clinical evidence by a considerable period of time. Fig. 1 shows the typical record of such a case. The patient had a sub-total hysterectomy in 1932 and was subsequently treated, in 1960, for a carcinoma of the

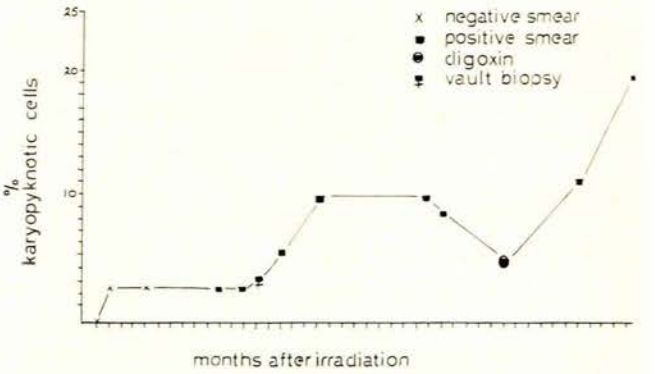

Fig. 1. Typical record of a patient with vault recurrence. KI complicated by the administration of a cardiac glucoside which may have had an oestrogen-like stimulation.

cervical stump. Follow-up smears were negative until February 1962 when cells which were interpreted as malignant were found. Vault biopsy in May 1962 was reported as 'malignant change restricted to the epithelium'. Further radiation treatment was given in June 1962, but her smears remained persistently positive until her death from coronary thrombosis in June 1965. Unfortunately no postmortem examination was performed so that further histological examination of the vaginal wall was not possible.

SUMMARY

In this brief review an attempt has been made to focus attention on the problems which may be encountered during the cytological follow-up of patients treated by ionizing radiation for malignant disease of the uterine cervix. It is suggested that profitable approaches to the problem of prognosis may be made by concentrating on the cytochemical and cytogenetic constituents of cervical tumours; and that the karyopyknotic index may prove to be an easily performed and reliable test for the early detection of recurrent carcinoma.

I am grateful to Prof. N. Sacks, Department of Radiology, University of Natal, and to the staff of the combined Gynae-

cology/Radiotherapy Clinic for their cooperation; and to the Chief Pathologist, Pathology Laboratory Services, Natal Provincial Administration, for permission to publish.

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