X-Ray Irradiation Induced Changes of the Nuclear Membrane of Kirkman-Robbins Tumour Cells

The nucleus can be considered the most radiosensitive component of cells[1]. In irradiated thymocytes the damage of the nuclear membrane is a primary phenomenon in a sequence of events leading to the dissolution of the nuclear chromatin[2,3]. The involvement of the nuclear membrane of tumour cells in the response to the ionizing radiation is unknown. To elucidate this problem we have undertaken an ultrastructural study on the changes induced in Kirkman-Robbins[4,5] tumour cells by X-rays. We describe in this communication in more detail the changes of the nuclear membrane and discuss their significance.

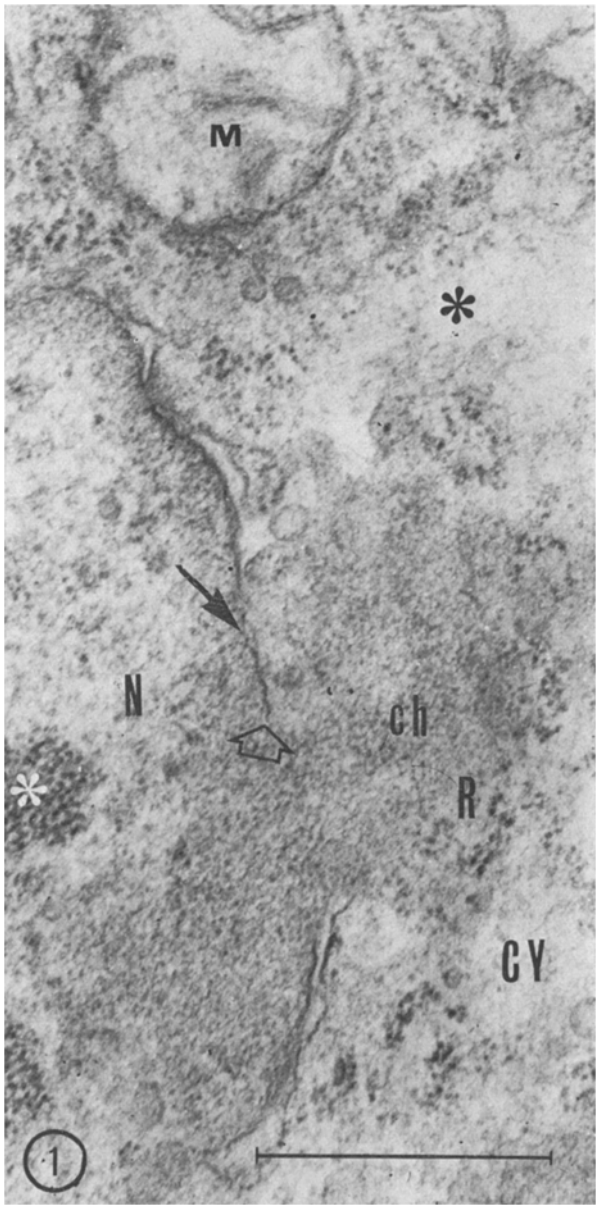
The inoculation of hamsters and the methods of preparation of tumour tissue for the electron microscope investigation were described previously[6,7]. The fixatives were instilled in situ before the samples of tumourt issue were collected. The tumour was in its 285th generation and was irradiated on 20th day after inoculation. We applied total body X-ray irradiation in a single dose of 2000 r at room temperature and normal air pressure to 3 hamsters carriers of Kirkman-Robbins tumour. The tumour was collected 24 h after irradiation. Non-irradiated tumour tissue was collected for comparitive purposes.

Observations of semi-thin sections of plastic embedded non-irradiated tumour cells revealed that all interphasic nuclei contained condensed chromatin and prominent nucleoli[7]. Irradiated tumour cells under light microscope showed a fragmentation of the chromatin and the loss of nucleoli. In some irradiated nuclei, the dissolution of the chromatin was also observed.

Electron microscope observations of non-irradiated interphasic tumour cells showed that the nuclear membrane, except for nuclear pores, was continuous over the whole nuclear perimeter; leaflets of the nuclear membrane were intact.

The fragmentation of internal mitochondrial membranes and dissolution of free polyribosomes were the most common cytoplasmic alterations induced by X-ray irradiation. The thinning, splitting and fragmentation of leaflets of the nuclear membrane with formation of blebs were the most characteristic alterations induced by irradiation in a great number of tumour nuclei. In some instances, the damage of the nuclear membrane was barely visible while in others completely broken nuclear membrane caused intermingling of nuclear and cytoplasmic components. An example of medium sized postirradiational damage of the nuclear membrane is presented in the Figure. The damage most frequently occurred in the vicinity of the nuclear pore. The structure of chromatin of irradiated cells was similar to that of non-irradiated cells. At the sites of the damage of the nuclear membrane, the chromatin left the nucleus and entered the cytoplasm and there intermingled with cytoplasmic polyribosomes. The nucleoli in a majority of irradiated cells are not visible. Aggregates of closely packed electron dense granules, probably remnants of nucleoli, were constantly found in the nuclear area.

The most significant postirradiational changes in Kirkman-Robbins tumour cells involved the structural damage of the nuclear membrane and the loss of typical nucleolar structure. The objection may be raised that the fragmentation of the nuclear membrane with displacement of the chromatin is an expression of a general fragility of the tumour tissue acquired as a consequence



Kirkman-Robbins tumour cell 24 h after X-ray irradiation with a single dose of 2000 r. Mitochondrion (M) contains damaged internal membranes. The zone around black asterisk shows dissolution of polyribosomes. In the nucleus (N) white asterisk marks an agglomerate of electron dense granules, probably remnants of a damaged nucleolus. The arrow shows the splitting of the internal leaflet of the nuclear membrane. Empty arrow shows the margin of the hole in the nuclear membrane bordered only by the internal leaflet. Nuclear chromatin (ch) which escaped through the hole in the nuclear membrane, is intermingled with the cytoplasmic polyribosomes (R). CY, cytoplasm. Scale marker = 0.5 μm. ×78,000.

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of X-ray irradiation, and does not constitute any specific alteration per se. This objection may be waved, however, on the basis of such alterations as the splitting and formation of blebs of the internal leaflet of the nuclear membrane. Moreover, the fixation in situ did not favour the mechanical damage of the cell due to the preparative procedure. The chromatin of irradiated KIRKMAN-ROBBINS tumour cells did not demonstrate any changes suggestive of the separation of histone from DNA contrary to findings on irradiated thymocytes[2,3]. It may be supposed that the irradiated chromatin of KIRKMAN-ROBBINS tumour cells remains intact because nuclear membrane of these cells lacks the respiratory enzymes, but we cannot furnish any experimental data for this assumption. It is noteworthy, however, that in tumour cells, as in other cellular systems[8], the primary target of the ionizing radiation is a nuclear membrane.

Riassunto. L'osservazione al microscopio elettronico del tumore di KIRKMAN-ROBBINS irradiato con raggi X dimostrava danni nucleolari, frammentazione, assottigliamento e scollamento dei foglietti della carioteca. Inoltre, nel citoplasma si osservava cromatina fuoriuscita dal nucleo.

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Autoradiographic Studies on the Rat Embryos in the Pre-implantation Stages

Although considerable attempts have been made to investigate the metabolic process of the early mammalian embryos in the preimplantation stage, our understanding of the events is rather fragmentary[1,2]. The present investigation was designed to explore autoradiographically the pattern of nucleic acid synthesis in the blastocyst during normal and delayed implantation stages in the rat. The rats used were adult virgin females of the Wister Imamichi strain ranging in weight from 180 to 240 g. Cyclic females in pro-estrus were caged with normal males and left overnight. Delayed implantation was induced following the method of COCHRANE and MEYER[3]. On the third day of pregnancy, mated female rats were bilaterally ovariectomized and each rat was treated with 4 mg of progesterone subcutaneously per day. The precursor was instilled directly into the uterine lumen 15 min before autopsy from the tubal end. The precursor used was ^{3}H-uridine (specific activity 5.0 c/mmole, Daiichi Kagaku Yakuhin K.K.; 5 μc/uterine horn in 0.05 ml sterile physiological saline). In the course of normal pregnancy, rats were autopsied 15 min after instillation of the precursor on the fifth or sixth day of pregnancy. In the delayed implantation the precursor was instilled into the uterine lumen of the ninth day of delayed implantation and in another group, 1 μg of estradiol-17β was injected s.c. on the eight day of delayed implantation and after 16 h the precursor was instilled into the uterine lumen. 2 or 3 blastocysts were together transferred to a slide glass and squashed gently with a cover slide. After the slides were rapidly frozen in liquid

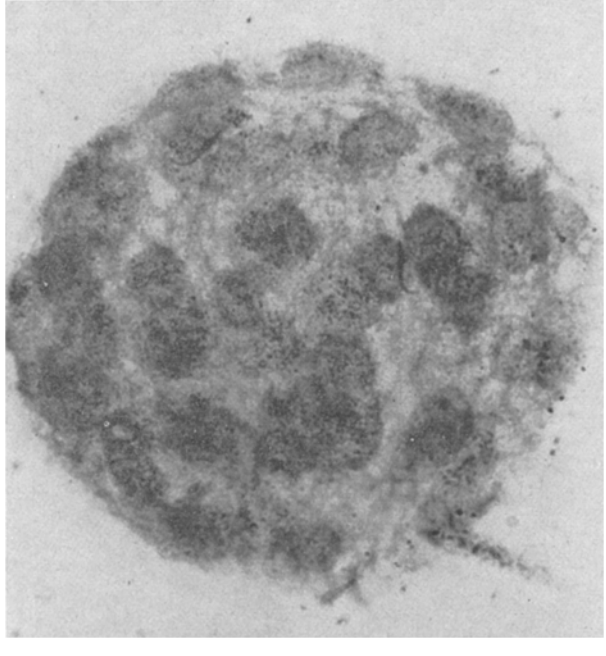

Fig. 1. Blastocyst of rat on day 5 showing the incorporation of ^{3}H-uridine. ×600.

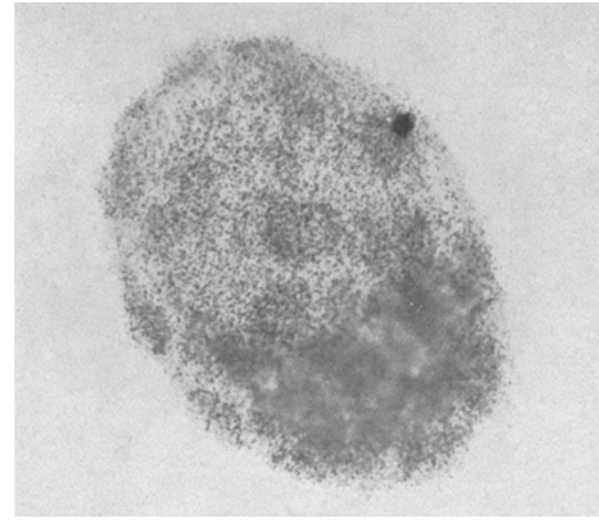

Fig. 2. Blastocyst of rat on day 5 showing the incorporation of ^{3}H-phenylalanine uniformly in all regions. ×600.

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