Polyploidy Induced by X-rays in Chinese Hamster Cells in vitro

Abstract. *The incidence of polyploidy (specifically, in this case, the doubling of the stemline chromosome number) induced by x-rays in cells cultured in vitro is about the same for both "near-diploid" and "near-tetraploid" sublines of the Chinese hamster. Endoreduplication is a principal cytological mechanism for this form of induced polyploidy.*

An increased percentage of polyploid cells has been observed in cell populations in vitro after they have been exposed to x-rays (*1*), especially when large doses have been given (*2*). On the assumption that the formation of polyploid cells (and in particular, cells of double the aneuploid stemline number) may be due to the same process irrespective of the ploidy of the irradiated cells, we have attempted to establish a dose response for the formation of "near-tetraploid" cells from "near-diploid" cells in culture and to compare this with the dose response for the formation of "near-octoploid" from "near-tetraploid" cells. Cells from the Chinese hamster, *Cricetulus griseus*, were used in this study. The incidence of higher polyploid forms (greater than twice the stemline number) induced by x-rays, will be discussed elsewhere (*3*).

Two cell sublines were used, which for convenience are referred to here as diploid and tetraploid, respectively, rather than "near-diploid" or "near-tetraploid." The diploid $122D_1$ (23 chromosomes) and the tetraploid 122T (46 chromosomes) were subcultured from the V79 clone (*4, 5*) of lung cells from a Chinese hamster (*6*). The nature, characteristics, and histories of these sublines were previously described by Yu and Sinclair (*7*). We grew the cells at 37°C in a humid atmosphere of 2 percent CO_2 and air, using HUT-15 medium similar to that of Elkind and Sutton (*4*). The cells were irradiated in plastic Petri dishes with 250 kv peak x-radiation (half-value-layer of the order of 0.9 mm Cu) at about 110 r/min. Cell samples of both lines were exposed to 0, 250, 500, 1000, and 1500 r. (The absorbed dose may be found by multiplying the exposures by 0.945 rad/r.) Chromosomes were observed in three experiments at intervals between 8 and 146 hours after irradiation. To each sample of cells, colchicine was added to a concentration of 2 μg per milliliter of medium for 2 hours. Cells were harvested with a rubber spatula, treated with hypotonic solution (0.9 percent sodium citrate), fixed in acetic-alcohol (1:3 by volume), air dried, and stained with 2 percent aceto-orcein.

The recognition of tetraploid and octoploid cells (that is, cells with double the stemline number) obtained in diploid and tetraploid cell lines, respectively, was accomplished by counting centromeres rather than chromosomes.

The percentages of tetraploid mitotic cells found in a preliminary experiment with the diploid subline at three different times after irradiation are shown in Table 1. This experiment showed that the percentage of tetraploid cells began to increase significantly at the 24-hour assay time and was still rising at 48 hours. A second experiment conducted over longer times showed that the percentage of tetraploid cells reached a maximum between 48 and 74 hours (Fig. 1). The number of mitotic cells examined varied, as shown, for example, in Table 1, mainly because of the range of mitotic indices in the samples studied. Small numbers of cells (100) were used only when the percentage of tetraploid cells was high.

In a third experiment, the percentage of octoploid mitotic cells (in the tetraploid cell subline) was studied at intervals between 24 and 144 hours. The results shown in Fig. 2 are similar to those obtained from the diploid subline—that is, the highest incidence of octoploid cells was found between 48 and 72 hours. However, below 500 r, the highest percentage of octoploid cells was obtained between 72 and 96 hours after irradiation. This was probably the result of an artifact in this particular experiment at 96 hours (a higher incidence of octoploidy was also observed in the control at this time). Even allowing for this, there appears to be some difference between the time relationships of the maxima of Figs. 1 and 2 at lower doses, which may be due to the dose response for division delay in the octoploid cells scored in Fig. 2 being different from that of the tetraploid cells scored in Fig. 1. We have no further information concerning this, however.

After the maximum was reached the incidence of polyploids began to decline, probably because the stemline cells in the population grew a little more rapidly than the polyploid cells. It is also possible that, ultimately, fewer polyploid cells survived than stemline cells. However, in experiments by one of us (W.K.S.) (*8*), in which clones surviving 1500 r delivered to single diploid cells were examined, between 30 percent and 50 percent of the clones were tetraploid, indicating that the survival of tetraploids was not much, if at all, inferior to that of the diploids (see Table 1 in *8*). This agreement also indicates that the incidence of polyploid cells in the mitotic population examined here is quite similar to the incidence of polyploid cells in the whole population.

The incidence of polyploids of greater than double the stemline number (*3*), which will be considered elsewhere, increased with time, reaching quite high values at later times of assay for the tetraploid subline (*3*).

The diploid and tetraploid cell sublines reached similar maximum values (for example, almost 50 percent cells of double the stemline number at 1500 r) between 48 and 74 hours (although this would have been more certain, for the diploid, if an additional sampling time between 74 hours and 146 hours had been included). The maximum incidence of polyploidy plotted against the dose is shown in Fig. 3. The diploid and tetraploid sublines gave about the same response within experimental error. The observed

Table 1. Percentage of tetraploid cells* obtained in a diploid cell subline of the Chinese hamster studied at intervals between 12 and 48 hours after irradiation.

Time after irradiation, (hr)	X-ray dose (r)				
	0	250	500	1000	1500
12	2.4 (500)†	1.0 (1000)	1.0 (500)	0.8 (500)	1.1 (350)
24	1.4 (500)	1.8 (600)	3.7 (300)	6.0 (300)	17.0 (100)
48	3.0 (100)	3.0 (100)	11.0 (100)	21.0 (100)	46.0 (100)

* Tetraploid cells include all cells with approximately 46 chromosomes. † Numbers in parentheses represent mitotic cells scored.

Table 2. Percentages of octoploid cells obtained in a tetraploid cell subline from the Chinese hamster studied at intervals between 24 and 144 hours after irradiation.* (EDR, endoreduplication: diplochromosome cells.)

Time after irradiation (hr)	Dose in r														
	0			250			500			1000			1500		
	8N†	EDR	Total	8N†	EDR	Total	8N†	EDR	Total	8N†	EDR	Total	8N†	EDR	Total
24	0.7	0.3	1.0	0.8	3.2	4.0	0.5	7.0	7.5	0.0	10.0	10.0	0.0	14.0	14.0
30	.5	.5	1.0	1.0	1.0	2.0	1.0	4.0	5.0	2.0	10.0	12.0	3.0	16.0	19.0
48	.5	.0	0.5	2.0	0.0	2.0	4.0	1.5	5.5	8.5	6.5	15.0	25.0	23.0	48.0
72	.5	.5	1.0	5.0	0.0	5.0	7.5	1.5	9.0	18.5	5.5	24.0	33.0	8.0	41.0
96	6.0	.0	6.0	9.0	0.0	9.0	18.0	1.0	19.0	20.0	2.0	22.0	26.0	5.0	31.0
120	1.0	.0	1.0	4.0	0.0	4.0	6.0	0.0	6.0	12.0	1.0	13.0	28.0	0.0	28.0
144	1.0	.0	1.0	1.0	0.0	1.0	6.0	0.0	6.0				26.0	1.0	27.0

* Between 100 and 2000 mitotic cells were scored in this assay (depending on the mitotic index) in a pattern similar to that in Table 1. † "True" octoploid (number of chromosomes doubled).

response shown in Fig. 3 may not represent the true incidence of chromosome doubling among initially single cells because, while the cell is in the process of doubling *chromosomes* (for example, endoreduplication), the unaffected stemline cells are actually doubling in *number*. Suppose x percent of N cells exposed to a given x-ray dose later became polyploid, then $(100 - x)$ percent of these N cells maintain the stemline chromosome number. If it is assumed that division delay and other time response factors are the same for these two components, the cells with the stemline number of chromosomes must double in number while the polyploid cells are passing through a process of chromosome doubling without increasing in cell number. Thus, the observed incidence y percent, at some later time, is given by

$$\frac{y}{100} = \frac{(x/100)\,N}{[(100 - x/100)\,2 + (x/100)]\,N}$$

from which,

$$x = \frac{200\,y}{100 + y}$$

The true incidence x percent is therefore higher than the observed incidence y percent, particularly at lower doses, and the response of "x" against dose for the data of Fig. 3 is almost linear. At present there are too many uncertainties and too few data for us to be explicit about the form of this response.

Octoploid cells formed from the tetraploid-cell subline after exposure to x-rays were studied in detail. The octoploid cells are of two types, those with the "true" octoploid complement (number of chromosomes doubled) and those with the diplochromosome configuration of the parental number. The percentages of both types of octoploid cells are listed in Table 2. In the earlier hours of assay, the number of diplochromosome cells (endoreduplication or EDR) increased as the dosage increased. The highest yield of diplochromosome cells, except for those irradiated with 1500 r, was found at 24 hours and decreased thereafter, whereas "true" octoploid cells increased with time. At 1500 r, the highest percentage of diplochromosome cells was observed at 48 hours (Fig. 4). The decrease of diplochromosome cells and the increase of "true" octoploid cells between 48 to 72 hours, shown in Fig. 4, are presumably due to conversion of diplochromosome cells into "true" octoploid cells after further division. Levan and Hauschka (*9*) have considered the configuration of diplochromosomes at metaphase to be the result of endoreduplication. Such chromosomes do not move apart during the first division but persist as diplochromosomes until the second meta-

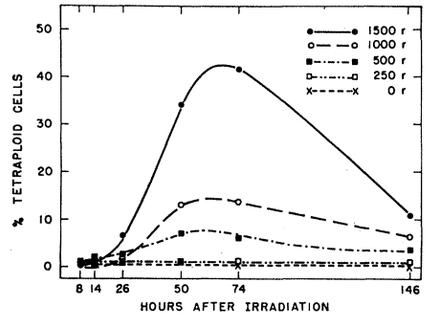

Fig. 1. The percentage of tetraploid cells induced in a diploid cell subline 8 to 74 hours after irradiation.

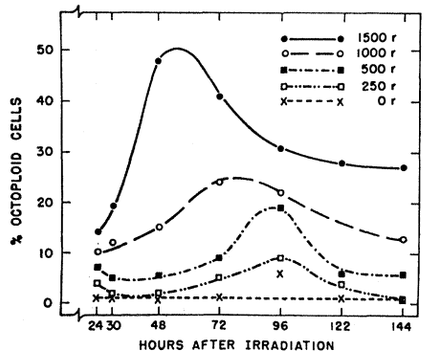

Fig. 2. The percentage of octoploid cells induced in a tetraploid cell subline 24 to 144 hours after irradiation.

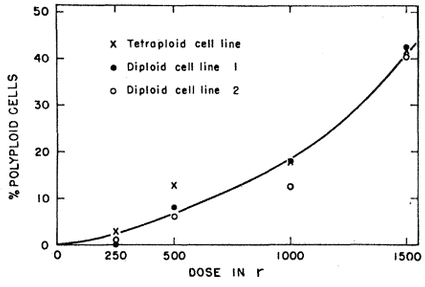

Fig. 3. Maximum percentage of polyploid cells (double the stemline number) plotted against the dose (corrected for control values).

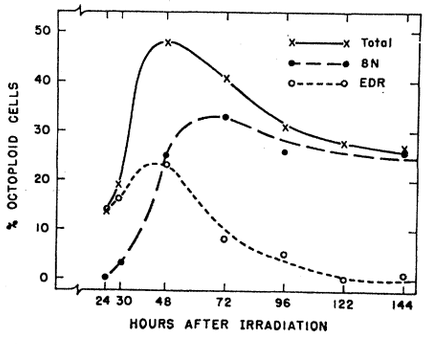

Fig. 4. Comparison of the percentage of "true" octoploid cells ($8N$) and percentage of endoreduplication (EDR) cells induced in a tetraploid cell subline at 1500 r plotted against the time after irradiation.

phase, when the cell appears as a "true" polyploid (*10*).

Endoreduplication therefore appears to be a principal mechanism responsible for the doubling of the stemline chromosome number. However, whatever the mechanism responsible for chromosome doubling, the form and time course of the process when induced by x-rays appears to be the same for diploid and tetraploid cells.

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Congenital Anomalies Induced in Hamster Embryos with H-1 Virus

Abstract. *Intravenous injection of H-1 virus into pregnant hamsters early in gestation produces an embryocidal and teratogenic effect. The congenital malformations, the presence of inclusion bodies in the fetuses, and the fact that the maternal animals are not affected by this virus, are points of similarity to the teratogenic effects of rubella virus and cytomegalic-inclusion disease virus in man.*

This report describes congenital malformations and fetal deaths induced by the intravenous inoculation of H-1 virus (*1*, *2*) into pregnant hamsters. To our knowledge only four viruses have been reported to have an etiologic relation to fetal defects in mammals. These include two agents causing congenital malformations in man, the rubella virus (*3*) and the cytomegalic inclusion virus (*4*). The other two viruses, associated with the use of vaccines in domestic animals, are the attenuated hog cholera virus vaccine which has caused edema and limb malformations in newborn pigs (*5*) and a modified blue-tongue live-virus vaccine used in pregnant sheep (*6*). Sheep so inoculated produced stillborn lambs or lambs which showed symptoms of central nervous system disease. It is difficult to ascertain whether lesions in the newborn pigs or lambs were true congenital malformations due to direct action of a teratogenic virus or whether they represented anomalies due to secondary effects of the immunization procedure.

Table 1. Effects of intravenous injection of H-1 virus on embryonic mortality in the pregnant golden hamster.

Gestation (day)	Vaccine *	Mothers treated (No.)	Fetuses (No.)		
			Living	Abnormal	Dying or resorbed
6	a	6	8	6	81
6	b	8	54	42	45
6	c	3	21	11	5
7	a	5	14	11	46
7	b	1	3	3	10
7	c	1	9	2	3
8	a	1	0	0	10
10–12	a	6	38	‡	24

* a, Undiluted tissue culture virus (see text); b, 10^{-1} dilution; c, 10^{-2} dilution. † Malformed or stunted (see text). ‡ All dead or dying; none malformed.

The H-1 virus was originally described by Toolan (*1*) and as used in our experiments had been carried through an unknown number of hamster passages. Stock preparations were obtained from virus propagated in rat embryo tissue cultures. The tissue culture methods used (*7*, *8*) are generally similar to those described by Moore (*9*).

Syrian golden hamsters were bred in the early evening hours, and the females were left with the males overnight. The day after breeding was counted as the first day of gestation, and the pregnant animals were caged individually, and fed diets of Purina lab chow. Pregnant hamsters (Table 1) were anesthetized with Nembutal (6.5 mg/100 g) after which 1.5 ml of a suspension of H-1 virus in tissue culture fluid was injected directly into the lingual vein (*10*) over a period of 1 minute. Animals were injected on either the 6th, 7th, 8th, 10th, or 12th days of gestation with identical volumes of either undiluted, or of 10^{-1} or 10^{-2} dilutions of the virus suspensions. On either the 10th or 13th day of gestation, the hamsters were lightly anesthetized with ether, and cardiac blood was drawn into a syringe containing heparin. Sterile specimens of uterus, placenta, and the corresponding fetus were collected and frozen at −40°C until the virus was titrated. For histologic examination corresponding specimens of uterus and placenta from the same animals, as well as specimens of maternal liver, kidney, and spleen, were fixed in Bouin's fluid. The embryos and fetuses were separated under a dissecting microscope and placed in alcohol-formalin-acetic acid fixative. Specimens from all maternal, placental, and uterine tissues were sectioned and stained with hematoxylin and eosin. Certain fetal specimens were sectioned and stained in a similar manner.

The maternal animals were watched daily after the injection of the H-1 virus. No signs of disease were noted. Maternal weight gain from the day of injection until the day the animals were killed was normal for pregnant animals, and histologic studies of sections of maternal kidney, liver, and spleen revealed no evidence of disease or of inclusion bodies.

The undiluted virus had a marked effect on embryos (Table 1), causing embryonic death in 2 to 4 days. In many of the litters it was not possible to determine the time of death, although in those considered to be recently dead or dying there was neither fetal motion on stimulation nor detectable circulation. Resorbed fetuses had placentas of normal size. An exencephalic fetus from a mother which received 1.5 ml of undiluted virus on the 7th day of gestation is shown in Fig. 1. Four surviving littermates had marked dilatation of the pericardial cavity; the pericardial cavity of some of these animals was filled with blood. Figure 2 represents another unusual malformation, lateral herniation of the liver—a common finding in animals whose mothers were treated on the 7th and 8th days of gestation. Other malformations noted in addition to these were facial clefts, facial asym-