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RADIATION-INDUCED ANEUPLOIDY IN THE MOUSE FETUS AND ITS POSSIBLE RELATION TO INCREMENTS IN CONGENITAL ANOMALIES*

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There is no longer any question but that ionizing radiations can have an immediate and morphologically damaging effect on mammalian chromosomes (Rugh, 1950). Such effects should be the more frequent and obvious in highly proliferating tissue such as that of the embryo or fetus. It is probable that the effect may involve both the dividing and the resting nuclei, only to be observed, however, in the condensed mitotic chromosomes. Thus, it may well be that the chromosomes at any phase of the mitotic cycle can be damaged even though they may not be discrete units at the time of bombardment. When chromomeres are dropped out the affected chromosomes do not readily pass to the spindle poles at anaphase but remain outside of the spindle possibly to form accessory micronuclei at telophase. These obvious deficiencies in the chromosomes carry with them genetic deficiencies which can manifest themselves, if they survive, in causing congenital anomalies in the resulting fetuses. It is therefore likely that when the deficiencies are of sufficient magnitude as to deter the normal ontogeny, the fetus will succumb. While these are conjectures, there is experimental basis for them and this study is designed to investigate the chromosomal changes that do occur at a gestational age and a dose level that has been previously demonstrated to induce congenital anomalies.

It seemed in order to determine (1) the normal variation in chromosome numbers of control fetal mice of 9 days gestation and (2) the immediate or delayed effects of fetal x-irradiation at various levels on such chromosomes, using doses known to cause congenital anomalies.

MATERIALS AND METHOD

CF1-S white female mice were time-mated with males of the same strain during a 45 minute period between 8 : 00 and 8 : 45 A.M. and those females exhibiting vaginal plugs were isolated for study. Exactly 9 days thereafter the pregnant females were given whole body x-irradiation at various rates from 10 to 400 Rpm and in doses from 100 R to 500 R, using 0.28 mm Cu and 0.50 mm Al filters, and parallel tubes with the

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pregnant females interposed equidistant between. At 4, 6, and 24 hours after exposure some of the pregnant mice were killed by cerebral dislocation and the fetuses removed. At 9 days gestation the fetuses are discernible under the dissecting microscope but are still very delicate and transparent so that whole embryos were dissected out for study. Other radiation factors were 184 kVp, 30 mA, HVL 0.6 mm lead.

The best technique for preparing the chromosome slides was a slight modification of that found in Ford & Woollam '63 with some of the changes suggested in a private communication from Dr. C. E. Ford. It follows :

The pregnant female was injected intraperitoneally with 0.3 ml of 0.025 % colchicine 1 to 1.5 hours before the intended sacrifice. This period can be lengthened a bit and might give more mitotic figures. Up to 12 days gestation the fetuses are small enough to be used whole. After that the fetuses may be removed, decapitated, and only their large livers may be used to give very satisfactory karyotype figures. Whether the whole fetus or the liver is used, it is placed in 5 ml of 0.1 % colchicine in Hank's balanced salt solution and broken up by gentle aspiration with a tapered pipette, using, of course, a different pipette for each sample. After 1 1/4 to 1 1/2 hours in suspension the whole is centrifuged for 5 minutes at 400 rpm and the supernautant fluid removed. Add 5 ml of 1 % sodium citrate and let suspension stand for 15-20 minutes (a longer time tends to cytolyze the cells). Centrifuge slowly for 2-3 minutes, to avoid damage to the cells. Add carefully 1 to 2 ml of fresh methanol and acetic acid (ratio 3 : 1), gently shake, and then fix at 4 °C for 30 minutes. Centrifuge, remove the supernatant fluid, add 1 to 2 ml of 45% acetic acid, centrifuge, and remove supernatant fluid. Add sufficient 45% acetic acid to make suspension for slides estimating about 0.5 ml for 6 slides. Pla ce drops of suspension on slides at 54 °C on a hot plate, 2 rows of 4 drops each. Each drop will form a series of concentric rings. When dry, stain in lactic-acid orcein (LAO) for at least 30 minutes, wash off excess stain with 3 changes of 45 % acetic acid, and air dry the slides. Since large aggregates of cells are often a problem, a very fine pointed pipette may be used, preferably one made from a 2 mm bore glass tubing drawn out in a flame to a fine point. Gently spread the cells in the drop.

The first series was made to determine the postradiation time at which the maximum number of damaged chromosomes might be found. Thus, 500 R was given, and the post-irradiation intervals were 4, 6, and 24 hours. Subsequently an exposure of 100 R was given but at different rates : 10 Rpm, 100 Rpm, and 400 Rpm and all embryos were examined at the same time, namely 6 hours post-irradiation. This was to determine whether dose rate would alter the incidence of chromosomal anomalies at the peak period of 6 hours after exposure.

EXPERIMENTAL DATA

The data can best be presented in tabular form (Table I). It will be noted that the greatest percentage of abnormal chromosomes was seen at 6 hours after exposure (65.7%) and the least at 24 hours after (29.1%), suggesting elimination of the abnormal

cells or, less likely, some sort of "recovery". It may also be noted that the evidence for some aneuploidy suggested by 2N + chromosomes, was greatest (15%) at 4 hours after elimination or "recovery". Finally, at 24 hours the normal set of 2N +* and the −2N some chromatic material were about equal to those showing damaged chromosomes.

Table 1. X-irradiation and fetal chromosomes in the mouse*

	CELLS	DAMAGED %	NORMAL 2N %	LESS THAN 2N %	MORE THAN 2N %
100R at 100Rpm+6Hrs.	(2516)	32.1	33.0	21.9	13.0
100R at 400Rpm+6Hrs.	(2607)	19.8	42.3	29.9	8.1
100R at 10Rpm+6Hrs.	(3023)	11.7	37.0	40.7	10.7
200R at 100Rpm+6Hrs.	(106)	17.0	38.7	13.27	31.1
500R at 250Rpm+4Hrs.	(110)	51.4	10.3	23.4	15.0
500R at 250Rpm+6Hrs.	(321)	65.7	12.5	15.0	6.9
500R at 250Rpm+24Hrs.	(103)	29.1	27.2	35.0	8.7
CONTROLS	(135)	0	74.1	12.6	13.3

* Pregnant female at 9 days gestation given whole body irradiation.
Number in parenthesis represents total karotypes counted.

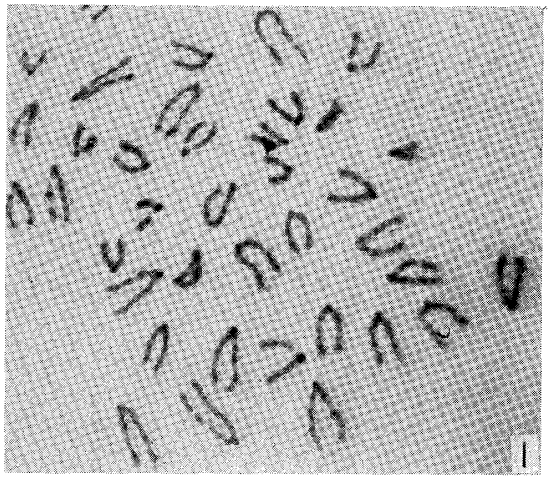
The rate data suggest that when examined at 6 hours after exposure the 100 Rpm rate causes more damage (32.1%) than either of the other rates, and the 10 Rpm the least, (11.7%). However, the lower rate (10 Rpm) showed the greatest numer of −2N counts (40.7%) and the greatest percentage of normals was found at 400 Rpm. This latter observation suggests that at the higher rate there may be some radiation wastage.

One must call attention to the controls in these studies for, while there were no cells with damaged chromosomes, there were indeed both 2N+ and −2N cells. Only 74.1% were entirely normal, with deviations both up and down in about equal numbers. The normal grouping of chromosomes meant 40, or 20 pairs and abnormal chromosomes generally meant obvious fragments and configurations not seen in the control material. Since the same technique was used for the control and x-irradiated fetuses, this possible source of damage or error can be ruled out by considering the control situation as the baseline.

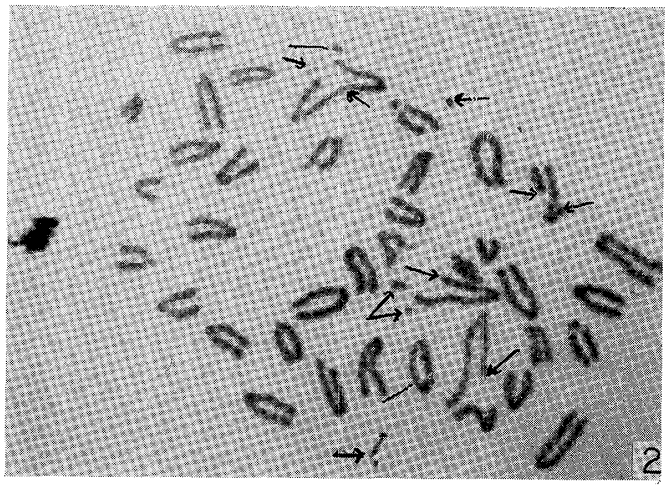
DISCUSSION

There is no longer any question but that ionizing radiation can have an immediate and damaging morphological effect on mammalian chromosomes, whether on *somatic*, tissue, (Bender 1964, Kaufmann 1954, Swanson and Johnson 1954, Carlson and Harrington 1955, Carlson 1958, Revell 1959, Bender and Wolff 1961, Chu et al. 1961, Buckton et al. 1962, Bender and Gooch 1963, Conen and Bell 1963, Sasaki et al.

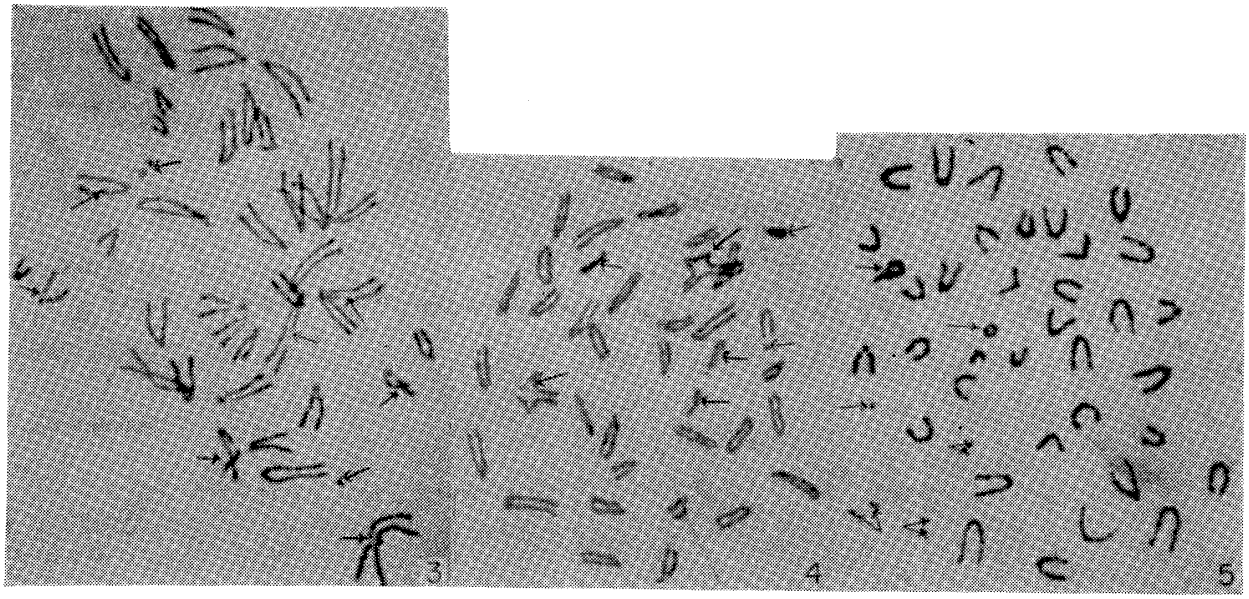
* (The use of 2N+ means a number in excess of the diploid or hyperdiploid 40 and − 2N means a total number of less than the diploid or hypodiploid number of chromosomes)



Control (40)



100 R + 6 hours (46)



500 R + 6 hours (41)

500 R + 6 hours (41)

500 R + 24 hours (43)

Fig. 1 Control cell showing forty normal chromosomes of 9 day fetus.

Fig. 2 Chromosomes of mouse fetus x-rayed at 9 days to 100 R and study made 6 hours later when maximum damage is expected. Fragmentation accounts for the count of 46 discrete units, but there are also some end-to-end fusions of chromosomes. Note deletions, double fragments and single chromatid breaks.

Fig. 3 Chromosomes of mouse fetus x-rayed at 9 days to 500 R and examined 6 hours later. Breaks, fusions, and double fragments may be seen, as well as deletions, all chromatid aberrations.

Fig. 4 Similar to Fig. 3, showing more fusions than breaks, as well as two quadri-radials. Several single chromatid breaks may be seen.

Fig. 5 Chromosomes of mouse fetus x-rayed to 500 R at 9 days and studied at 24 hours thereafter. Note double fragments, loops, folds, as well as loss of centromeres. These chromosomes appear to be hyperchromatic, and are likly moribund since this is a 100% lethal exposure level.

Note : It is likely that x-irradiation + 6 hours catches the figures in chromatid stage while at +12 hours they are more likely to be discrete chromosomes.

1963, Bloom and Tjio 1964, Buckton and Pike 1964, Nowell et al. 1964, Schmickel et al. 1964, Nowell et al. 1965, Wolff and Luippold 1965, Wolff 1965, Borstel and Miller 1966, Wolff 1967, Wolff 1968), on *tissue cultures* (Chu and Monesi 1960, Ohnuki et al. 1961, Stroud et al. 1962, Bell and Baker 1962, Visfeld 1963, Prokofeva-Bel govskaya 1964), on *germinal tissue* (Rugh 1950, Dekaban 1965) or on *embryos or fetuses* (Hungerford 1955, Carr 1963, Warkany et al. 1964, Boeck 1962, Carr 1965, Russell 1965, Soukup et al. 1965, Leonard and Deknudt 1967, Dekaban 1968, 1969).

Some studies have already suggested that chromosomal anomalies may be associated with congenital anomalies, and even be the cause of them (Ford et al. 1959 a and b, Turpin et al. 1959, Böök 1960 a and b, De Carli et al. 1960, Ferguson-Smith and Johnston 1960, Fraccaro et al. 1960, Fraser et al. 1960, Harnden et al. 1960, Lejeune et al. 1960, Patau et al. 1960, Puck 1960, Tough et al. 1960, Breg and Miller 1961, Lee et al. 1961, Butler et al. 1962, de Bellefeuille 1962, Grunbach et al. 1964, Koenig et al. 1962, Kowalczyk 1964, Lejeune et al. 1964, Macintyreet al. 1964, van der Hagen and Brogger 1965, Singh and Carr 1967).

Chromosome studies may be made on a qualitative or a quantitative basis, the former having genetic implications and interpretations while the latter may affect not only genetic consequences but the developmental processes. This study did take into account qualitative changes to the extent that there were fragmentations and configurations not seen in the controls, but these were categorized on a quantitative basis. For example, a 2N count might well be genetically abnormal but quantitatively normal, following deletions, additions, etc. which did not alter the total count. In the controls no damaged chromosomes were found, but there were a considerable number of cells which had 2N + or −2N chromosomes, and yet they were from a normally developing fetus. The shift, when one examines those that had been x-rayed, is toward actually damaged chromosomes with the least evidence of this when 100 R was given at the slowest rate. In this case (3,023 cells examined) 40.7% of the cells were hypodiploid. This reduction in number is in excess of the controls (12.6%) but still in a direction of proven viability. The minus factor need be only one chromosome, of course.

One would like to bridge the gap between the threshold dose of radiation in any. amount and at any given rate, and the development of a congenital anomaly which might be associated with that threshold radiation. But there are related variables such as oxygen concentration (Goulden et al. 1953) and the mitotic stage and DNA synthesis (Puck 1964) which alter the radiosensitivity of chromosomes. The degree of tolerance by embryonic cells of deviations from the 2N condition, both in quantity and quality, may be difficult to establish. It is one thing to determine the linear order of the genes, and the relation of genotype to phenotype, and yet another thing to link a specific chromosome with a developmental deviation. One should first establish the threshold of tolerance of chromosomal deviations in the embryo and work downward to those deviations that can be associated with a specific developmental anomaly.

This study seems to indicate two major findings: *First*, that chromosomal effects to be seen after whole body x-irradiation are at their maximum at about 6 hours after exposure, suggesting that (a) they may await the cleavage process or (b) the mitotic index is such that it takes this much time to accumulate a demonstrably significant number of chromosome figures. *Second*, it also shows that there seems to be a rather wide range of normality so that even the controls show frequent deviations from the 2N count without any evidence of gross effect on development. It would be of interest to determine to what extent survival of x-irradiated cells is due to the scavenging of cells so abnormal that they could not allow normal development, the result being an apparent recovery.

SUMMARY AND CONCLUSIONS

1. The highest incidence of obvious damage to the chromosomes in fetal cells is seen at about 6 hours after x-irradiation.
2. By 24 hours after x-irradiation there appears to be the lowest incidence of damaged chromosomes. This suggests that such cells may have been scavenged, by phagocytes.
3. Rate of exposure does seem to alter the incidence of damaged chromosomes. An exposure to 100 R x-rays at 100 Rpm showed the greatest percentage of damaged chromosomes. Possibly at the higher rate of 400 Rpm there was some radiation wastage and at 10 Rpm there could be some "recovery" and/or scavenging of cells.
4. The controls showed no damaged chromosomes while cells from every level and rate of x-irradiation did show chromosome damage. However, the controls did show some deviation from the diploid counts, both up and down.
5. Cells with less than the 2N count following x-irradiation were found in considerable numbers 24 hours after 500 R at 250 Rpm. This reduction may be due to some stickiness, fusing chromosomes to reduce apparent numbers.
6. Cells with 2N plus chromosomes were found most frequently 4 hours after 500 R at 250 Rpm which suggests immediate fragmentation of chromosomes.
7. It seems evident that x-irradiation effects cannot be judged completely at any one post-exposure time, for the factor of subsequent mitosis, scavenging, and possible repair or adhesion of chromosomes could alter the counts. No "recovery" is implied in the sense of re-joining of the fragments so that any particular chromosome is broken and then returns to its pre-fragmentation state. This is only theoretically possible, and not within the realm of statistical probability. Also it is still difficult to establish a direct causal effect of congenital anomolies associated with a specific chromosomal aberration since, in any litter, there is possible such a wide variety of congenital anomalies that can follow whole body exposure in a uniform field.

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