Environmental Health Perspectives
Vol. 31, pp 13-17, 1979

Radiation-Induced Nondisjunction

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The methodology and results of epidemiological studies of the effects of preconception diagnostic x-rays of the abdomen on chromosome segregation in humans are described. Many studies have been conducted in a number of different countries. The vast majority show the same positive, though not significant, trend to increased nondisjunction among the offspring of irradiated women. The results of the various studies, however, cannot be pooled because of differing methodologies used. A worldwide co-operative project with standardized methodology is recommended. Such a study should identify the parental origin of the nondisjunctional event before etiological factors are investigated. Abnormal chromosome segregation during mitotic division has been inducted experimentally by the *in vitro* exposure of human lymphocytes to a low dose of 50 R gamma irradiation. First meiotic nondisjunction has been successfully induced by whole body exposure of female mice to a low dose of radiation. Further experiments are being conducted to try to induce abnormal segregation during second meiotic division. Because of difficulties encountered in trying to estimate total gonad doses resulting from differing techniques employed by radiologists and other health personnel, no attempt has been made to estimate the doubling dose nor minimum safe dose regarding the effects of radiation on chromosome segregation in humans. The question of time-related repair of the mechanism involved in chromosome segregation is raised.

Some 7-8% of detectable human conceptions have chromosome aberrations, most of which are aneuploids. Very few are segregation products of parental balanced translocations. Although the vast majority are spontaneously aborted, these abnormalities represent a high toll of human life. About 1% survive the first two trimesters but many die before or during the neonatal period. Although aneuploidy may result from undetected mosaicism in a parent, environmental mutagens are suspect in the etiology because of increasing evidence that more aneuploids are being born to younger parents. This is contrary to the expectation that the frequency of aneuploids would fall with the increasing use of birth control pills among older mothers and therapeutic abortions of trisomics identified with amniocentesis.

The only feasible method of determining the harmful effects of environmental mutagens on the meiotic processes in women at present is by epidemiological studies. Over many years there has been spasmodic but insufficient interest in the possible effects of ionizing radiation as a cause of nondisjunction. Radiation has been known for many decades to cause gene mutations and chromosome breaks and rearrangements, but its effect upon chromosome segregation is still being questioned. Anxiety by the public is being expressed because of more frequent referrals for radiological examinations by physicians who fear litigations arising from missed diagnoses. X-ray equipment is being used not only by qualified radiologists but also by dentists, chiropractors, and even physicians in private offices who may ignore simple precautions to protect themselves and their patients. There is insufficient inspection of antiquated equipment. Attempts to meet the recent energy crisis with the greater use of nuclear energy is increasing public involvement in attempts to control environmental mutagens.

To examine the possible effect of radiation as a nondisjunction-inducing agent among humans, the obvious method of study is a retrospective survey, that is, to concentrate upon a specific disorder and examine the past history of the parents. Recently much attention has been centered around Downs' syndrome because of its high frequency among spontaneous abortions as well as live births and because the ability to identify the parental origin of the extra chromosome has revealed an unsuspected high frequency of paternal transmission.

The publication of Patterson et al. (*1*) pointing to a positive correlation between abnormal chromosome segregation and radiation exposure of aged

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Drosophila females stimulated our interest to examine a similar mechanism in humans. A preliminary investigation was conducted by mailing out questionnaires to a series of parents known to have children with Down's syndrome. The good response received led to a more formal project including control parents of children with isolated cleft lip and/or palate and personal interviews of all mothers participating in the study. Detailed questioning of parents revealed more histories of radiation exposure, particularly fluoroscopic examinations, than had been recorded on the questionnaire. Results of this study suggested that several exposures to abdominal x-rays and/or fluoroscopy before conception could increase the frequency of Down's syndrome by a factor of four (*2*).

Because a retrospective survey is dependent upon the mothers' power of recall, more accurate information should be available by carrying out a prospective study. The names of women as they report for diagnostic x-ray examinations could be obtained and the women followed over many years to record the type of children born subsequent to the radiation exposure. Comparisons could then be made with controls consisting of offspring of age-matched unirradiated mothers. This method, however, involves a long latent waiting period.

A more rapid, if not less difficult, method was decided upon (*3*). The names of women previously exposed to abdominal x-rays were obtained from the radiology department of a large hospital. The type of x-ray examinations and estimated gonadal doses are given in Table 1. A search was then made to find these women to see what kind of children they had produced during the intervening years. Some of the problems encountered were difficulties in locating the families, temporary or permanent sterility caused by radiation exposure, no subsequent offspring born to older women, inclusion of unmarried women with no progeny, and neonatal deaths before karyotyping could be performed. Choice of suitable controls presented a major hurdle until it was decided to use the children born to these same women before x-ray exposure, thus minimizing genetic and constitutional differences. This method also reduced the number of families to be interviewed. The women were cross-matched with each other for maternal age. This survey of 972 progeny in each group revealed 10 trisomics born to irradiated women compared with one in the nonirradiated sample. The results were also consistent with increased radiosensitivity with late maternal age. Mean ages of the mothers at the time of the x-ray examinations and at childbirth subsequent to the exposure are given in Table 2 along with mean maternal ages of nonirradiated controls.

Table 1. Radiological examinations with estimated gonadal doses, 1956-59.

Examination	Dose, Mrads[a]
Abdomen re pregnancy	300
Placentogram	500
Pelvimetry	800
Gallbladder	100
Abdomen	150
Cholangiogram	400
Gastrointestinal series	700
Small-bowel series	1600
Barium enema	800
Double-contrast enema	1200
Intravenous pyelogram	1600
Salpingogram	400
Pelvis	500
Lumbar spine	800
Lumbar myelogram	2000
Discogram	2000
Skeletal survey	1000
Femur	350

[a]Doses estimated by Dr. M. K. Kiernan, radiologist, Winnipeg General Hospital.

Another productive method of obtaining information on radiation-induced nondisjunction makes use of spontaneous abortions. Up to 50% of the products of conceptions spontaneously aborted during the first trimester are chromosomally abnormal (*4*), the vast majority being aneuploids. In the study by Alberman and her colleagues (*5*), the mothers of

Table 2. Mean maternal ages at birth of children and at time of x-ray exposure.

	Control		Irradiated		
Type of offspring	No. children	Maternal age at birth, yr	No. children	Maternal age at birth, yr	Age at exposure, yr
Total sample	972	30	972	30	27
Stillbirths	34	33	20	36	33
Neonatal deaths	19	30	18	32	30
Congenital anomalies	29	29	32	30	27
Aneuploids	1	38	10	36	33

Table 3. Studies of maternal radiation exposure before conception of aneuploid progeny.

Study (country, year)	Association[a]	Significance[b]
Lunn, Scotland, 1959 (*6*)	+	NS
Uchida and Curtis, Canada, 1961 (*2*)	+	S
Carter et al., England, 1961 (*7*)	−	NS
Stevenson and Matousek, Iceland, 1961 (*8*)	+	NS
Schull and Neel, Japan, 1962 (*9*)[c]	−	NS
Sigler et al., U.S., 1965 (*10*)	+	S
Uchida et al., Canada, 1968 (*3*)[c]	+	S
Marmol et al., U.S., 1969 (*11*)	+	NS
Stevenson et al., England, 1970 (*12*)[c]	+	NS
Villumsen, Denmark, 1970 (*13*)	+	NS
Alberman et al., England, 1972 (*14*)	+	S
Alberman et al., England, 1972 (*5*)	+	S
Boué et al., France, 1975 (*4*)	+	NS
Awa, Japan, 1975 (*15*)[c]	+	NS
Kochupillai et al., India 1976 (*16*)	+	S
Cohen et al., U.S., 1977 (*17*)	−	NS

[a]+ = more aneuploids among progeny of irradiated than nonirradiated mothers; − = fewer aneuploids among progeny of irradiated mothers.
[b]S = significant; NS = not significant.
[c]Prospective studies.

chromosomally abnormal fetuses had received significantly more preconception radiation than the mothers of chromosomally normal fetuses who in turn had had more exposures than mothers of live births. Attempts were made to obtain paternal radiation histories but the information was too incomplete to be informative. Boué et al. (*4*), on the other hand, found a significantly higher frequency of occupational exposure to radiation among the fathers of abnormal fetuses.

Epidemiological studies of radiation-induced nondisjunction have been undertaken by a number of investigators in many countries (Table 3), most of which demonstrate the same positive trend, though in many the increase is not significant. Of the four prospective studies, only one population, all victims of the atomic bomb in Japan (*9*), shows a lower frequency of Down's syndrome among the progeny of the exposed subjects. Later karyotyping of the same population revealed a number of sex chromosome trisomics (*15*). The significant increase of x-ray exposure of mothers of Down's syndrome children reported in 1965 by Sigler et al. (*10*) was later found to be reversed (*14*), i.e., more maternal radiation histories among controls than affected, when the original sample drawn over more than 16 years was increased by the addition of seven years. A particularly relevant investigation undertaken in Kerala (*16*), an area famous for its high background radiation, revealed a marked prevalence of Down's syndrome as well as other abnormalities.

Because of insufficient data, epidemiological studies of nondisjunction among humans cannot be carried out on a scale sufficiently large by any one group to produce convincing results. Unfortunately it is impossible to pool the results of the many studies already reported because of differing methodologies employed, particularly in the type of controls used and the method of ascertainment of radiation histories. For controls some investigators used age-matched mothers, others did not; some used mothers with abnormal offspring, others used normals; some made comparisons with expected frequencies based upon population incidences. In the ascertainment of radiation histories, a great deal of prompting is required. The method of ferretting out information needs to be standardized with the use of the same leading questions, such as: Were there any problems with digestion? What about the kidneys? Were chiropractors consulted because of back aches? Was there any exposure to occupational hazards? Were pre-employment x-rays required for workmen's compensation records?

Following the identification of inherited variants with a variety of chromosome banding techniques, a surprising number of instances of paternal transmission of the extra chromosome in Down's syndrome has been detected. Up to one third of affected children are the result of abnormal chromosome segregation in the father (*18*). The presence of inherited variants may also indicate the stage of meiosis when misdivision occurred. Most segregation errors occur during first meiotic division (*18, 19*). The problem as to whether or not the father's age is associated with

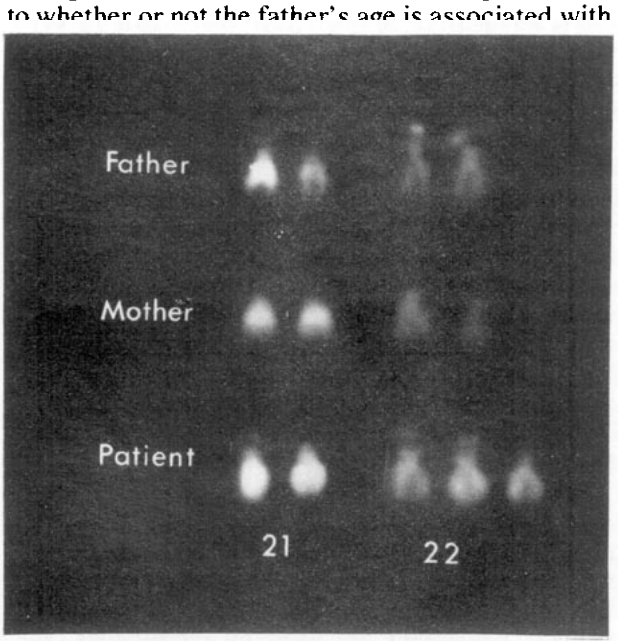

FIGURE 1. G group chromosomes from an abortus illustrating paternal transmission of an extra No. 22. Time of the meiotic error cannot be determined.

paternal nondisjunction has yet to be resolved. The apparent decrease in mean maternal age noted in Down's syndrome (*20, 21*) may be the result of increasing paternal nondisjunction. Former studies with their emphasis on the mothers may not be completely invalidated because of the undoubted association with late maternal age in the past. However, it is imperative that future investigations identify first the origin of the nondisjunction event (Fig. 1) before mutagenic agents are evaluated. In studies now underway in our laboratory the data are as yet too sparse to draw any conclusions but there appears to be a trend to higher frequencies of x-ray exposure in the parent who had contributed the extra chromosome in affected children as well as in spontaneous abortions.

In humans mitotic nondisjunction has been induced experimentally *in vitro* by irradiating lymphocytes as well as exposing unirradiated lymphocytes to irradiated serum (*22*). The radiation dose was kept at 50 R to minimize chromosome breaks, and the specimens were cultured for 72 hr to obtain cells in second metaphase. The frequencies of trisomy were significantly increased in irradiated cells and also in nonirradiated cells exposed to irradiated fluid. The increases were due to abnormal segregation of chromosomes 21 and X.

Preliminary experiments have also been undertaken to test the effects of a higher dose of 500 R. There was no increase in trisomy compared with the frequency found with 50 R. However, as anticipated, the frequency of chromosome breaks was significantly increased with the higher dose when the cells were irradiated. No correlation between increased dosage and frequency of breaks was noted when only the fluid was irradiated.

Other experimental studies have been carried out with mouse oocytes but this aspect will be covered later by others in this workshop. Suffice it to say that nondisjunction was induced during first meiotic division of oocyte chromosomes of irradiated young and aged animals (*23, 24*). Similar experiments testing for second meiotic nondisjunction are now underway with the use of preimplantation embryos. In all these experiments, it was essential to use a dose low enough to minimize chromosome breaks and yet high enough to induce misdivision: a level of 10-30 R was chosen.

In no human studies has there been an attempt to estimate the minimum allowable dose, if any, to avoid segregation abnormalities. The average gonad dose received by mothers of abnormal progeny is difficult to estimate because different methods and different equipment are used by radiologists, the number of original and repeat exposures are not always recorded, previous exposures at other hospitals are not remembered or records have been destroyed, exposure time during fluoroscopy varies considerably and, in addition, there is no standardization of methods used by non-radiologists.

Another problem is that of the time interval between radiation exposure and conception of aneuploid progeny. There may be a span of a few days to several years. An experiment has been initiated with the use of an animal model to examine possible repair of the mechanism responsible for abnormal segregation.

Investigation of environmental factors is a timely topic because of the technological advances that may add to higher standards of living but increase our load of harmful mutagens. With better understanding of many disease processes and the technological advances that increase survival, the chromosomally abnormal child is becoming a major problem in many branches of medicine because of the increasing costs of medical, institutional, and home care as well as the psychological burden among members of affected families.

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