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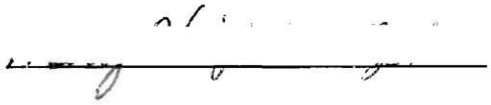

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CHROMOSOMAL ABERRATIONS IN THE CELLS OF CHINESE HAMSTER (*CRICETULUS GRISEUS* MILNE-EDW.) EMBRYOS AND OFFSPRING AFTER PATERNAL X-IRRADIATION

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CHROMOSOMAL ABERRATIONS IN THE CELLS OF CHINESE HAMSTER

(*CRICETULUS GRISEUS* MILNE-EDW.) EMBRYOS

AND OFFSPRING AFTER PATERNAL X-IRRADIATION

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SUMMARY

The object of this study is to determine whether acute whole-body X-irradiation of the male Chinese hamster, Cricetulus griseus Milne-Edw., results in the transmission of cytologically observable aberrations to embryos and offspring of matings with non-irradiated females. Adult males were irradiated with 400 roentgens of 250 KV X-rays delivered at 80 roentgens per minute and then mated during the presterile period with proven females. In one group gravid females were sacrificed after 15 days and individual embryos were minced and cultured in vitro; in the other group females were allowed to deliver their offspring which were subsequently sacrificed at one month of age, and their lungs were minced and cultured in vitro.

When cell growth was sufficient, slide preparations were made for metaphase analysis. Thirty cells per slide were examined for chromosomal abnormalities, using phase contrast microscopy. Five cells from each slide were photographed for more detailed karyotype analysis: measurements were made on the two largest pairs of chromosomes, and the centromere index and homologue ratio were calculated.

No aberrations were found in the control karyotypes. The karyotype of one cultured embryo sired by an X-irradiated male revealed the presence of a reciprocal translocation which was present in all thirty cells examined. The karyotype of a lung culture of a month-old offspring of an X-irradiated male revealed the presence of a reciprocal translocation and

a missing chromosome; these anomalies were seen in all thirty cells examined. Since these anomalies are found only rarely in normal somatic cells and since no aberrations were found in our controls, it is concluded that they are the result of radiation-induced damage to the male gametes. Statistical analysis of the measurements made on the five cells of each karyotype revealed the presence of small differences in the morphology of chromosomes in several cases.

CHAPTER I

INTRODUCTION

Radiation-induced chromosomal aberrations at the first metaphase after irradiation are studied extensively by the radiation cytologist. The radiation geneticist surveys cell populations or organisms, which are removed from the initial radiation event by many cell divisions or subsequent meiotic divisions, for genetic effects of aberrations and rarely observes the actual chromosomal aberrations. Since the aberrations scored by the cytologist are the ones most likely to produce mitotic inactivation or cell reproductive death in the carrier cell or its progeny, the cytologist has a rather artificial view of the importance of radiation damage (Lindsley, 1963). It would be informative to determine the persistence of aberrations produced by radiation in male gametes at the earliest stage at which chromosomal aberrations are cytologically analyzable. The object of this study is to determine whether acute whole-body X-irradiation of the male Chinese hamster, Cricetulus griseus Milne-Edw., results in the transmission of cytologically analyzable aberrations to embryos and offspring of matings with non-irradiated females.

The only mammalian genus used to any extent in radiogenetic studies is the mouse; the limited amount of work done on the rabbit, guinea pig, and rat has confirmed the findings in the mouse (Russell, 1954; Mandl, 1964). Since our experiments are concerned with the effects of acute, paternal X-irradiation of the Chinese hamster, the following discussion

will be limited to these effects in mammals. The effects of paternal X-irradiation on reproduction are manifest at various levels, among which are effects on (1) the ability of the irradiated animal to produce viable gametes, (2) the ability of irradiated gametes to accomplish fertilization, (3) the ability of gametes after fertilization to yield a viable zygote, and (4) the ability of the zygote to mature and produce viable offspring (Mandl, 1964).

Several investigators have studied the fertility of males (mice, rats, and guinea pigs) after an acute exposure to high intensity X-rays: Russell has summarized their results for exposures in the range of 400-1000 r. In this range there is an initial period of fertility (the presterile period), the length of which depends on the species and other factors, followed by a period of infertility the duration of which depends on the exposure. Finally there is a resumption of fertility, i.e., the poststerile period (Russell, 1954). The amount of exposure up to 800 r has been shown not to have any effect on the rate of spermiogenesis in mice (Hertwig, 1938; Eschenbrenner and Miller, 1950).

The initial depletion and eventual recovery of the seminiferous epithelium after acute X-irradiation has been demonstrated to result from the response of the spermatogonia to irradiation (Hertwig, 1938; Eschenbrenner and Miller, 1950; Oakberg, 1957). Hertwig (1938) examined histologically the testes of mice given 800 r and determined the reactions of the various types of spermatogonia. The "bright dusty" spermatogonia were always present, and she concluded that rebuilding progressed from them. The "crusty" spermatogonia disappeared first (within 24 hours)

along with spermatogonial mitoses. The "dark dusty" spermatogonia disappeared after the "crusty"; she assumed that these were the direct predecessors of the spermatocytes. Eschenbrenner and Miller (1950) found no direct evidence for cell death and concluded that inhibition of spermatogonial mitoses led to the depopulation of the germinal epithelium in the mouse. Oakberg (1957, 1971) found in the mouse that intermediate and type-B ("crusty") spermatogonia are extremely sensitive to killing whereas type-A ("dusty") spermatogonia possess a heterogeneous sensitivity; a few type-A spermatogonia ("bright dusty") survive and eventually repopulate the seminiferous epithelium. Jones (1960) discovered in the rat a similar heterogeneous sensitivity of the type-A spermatogonia, but he also found that the rate of depletion and subsequent recovery of spermatogonia were slower and the incidence of degenerating cells was lower in the rat than in the mouse. He concluded that the depletion of the spermatogonia in the rat may be due primarily to the inhibition of mitoses of some type-A spermatogonia rather than to cell death of spermatogonia as in the mouse. Thus, in spite of the extreme sensitivity of spermatogonia to X-irradiation, the eventual return to fertility can be explained in terms of the heterogeneity of the type-A spermatogonial population (Russell and Saylors, 1963).

The later stages of spermatogenesis (spermatocytes, spermatids, and spermatozoa) disappear in the order of their formation from spermatogonia (Hertwig, 1938; Eschenbrenner and Miller, 1950). Spermatocytes and spermatids present at the time of irradiation complete their development, and with the spermatozoa, insure an initial period of fertility in the mouse

(Eschenbrenner and Miller, 1950). With relatively high doses in mice, spermatocytes do not survive and the presterile period is reduced (Russell and Saylors, 1963; Mandl, 1964).

Spermatids and spermatozoa have been found to be extremely resistant to cell-killing by X-irradiation (Mandl, 1964; Russell and Saylors, 1963). No effect on the viability or motility of mature spermatozoa has been observed as a result of X-irradiation at exposure levels practical for breeding experiments (Russell, 1954). With exposures to rabbit spermatozoa (in vitro) as high as 6500 r, no effect was found on motility, viability, and ability to penetrate and activate the ovum (Amoroso and Parkes, 1947; Chang, Hunt, Romanoff, 1957). Henson (1942) X-irradiated rats (100-1000 r) and observed similar results. Strandskov (1932) irradiated guinea pigs at 84.6-2590.0 r and found numerous motile spermatozoa and assumed, without proof, that fertilization was normal. Russell summarized the work of additional investigators who found no effect on the viability, motility, and fertilizing ability at exposures of up to 400 r for the mouse and 1800 r for the rat (Russell, 1954).

Whereas irradiated spermatozoa may retain their motility and ability to initiate fertilization (penetration and activation of the ovum), they may not be able to complete fertilization (pronucleus formation and syngamy); activated ova may fail to divide (Chang, Hunt, and Romanoff, 1957). Amoroso and Parkes (1947) found that with high exposures (1000 r) to rabbit spermatozoa, the male pronucleus was abnormal, syngamy was delayed and irregular, and many activated ova failed to divide. Hertwig (1935) found that ova in the fallopian tube of female mice 24 hours after mating with irradiated males (1400-2200 r) exhibited frequent lagging

chromosomes and elimination of the male genome. Ova in which syngamy was incomplete failed to develop beyond the blastocyst stage (Hertwig, 1935).

Hertwig and Brenneke (1937) found that a number of the normal appearing "eggs" die after implantation. Regaud and Dubreuil (1908) reported the resorption of abnormal embryos sired by male rabbits exposed to X-rays. Henson (1942) also found evidence for resorption of embryos in the rat after exposure of the male to over 1000 r. Strandskov (1932) found that litter size was reduced for X-irradiated male guinea pigs and postulated that this was due to resorption during pregnancy. Russell (1954) found that in mice an exposure of 800 r resulted in the death of 38 percent of the embryos before implantation; he postulated from his data, and that of others, that the effect of raising the exposure is to increase the ratio of preimplantation to postimplantation deaths. Lyon and Smith (1971) contend that there are genera differences in this ratio; whereas in the rabbit the major part of embryonic death occurs before implantation, in the rat, mouse, guinea pig, and golden hamsters, the major part is after implantation for a single dose. Thus arrested development of tubal ova and resorption during pregnancy result in the lowering of litter size.

These embryonic effects have been interpreted as the result of the induction of dominant lethal mutations because of the appearance of lethality in the F_1 generation. Direct evidence that these dominant lethal mutations are chromosomal aberrations such as rings, dicentrics, and deletions has not been obtained. Indirect evidence (lagging chromosomes, supernumerary nuclei, inhibition of mitosis), however, points to chromosomal aberrations as the probable cause (Hertwig and Brenneke, 1937;

Russell, 1954).

Development in mammals does not proceed unless a nearly complete diploid chromosome set is present. Amoroso and Parkes (1947) and Hertwig (1935) found that activated ova, which were haploid because of the failure of the fusion of the male and female pronuclei, did not develop. Edwards (1954) demonstrated that increasing X-irradiation (up to 50,000 r) to mouse spermatozoa resulted in the production of embryos with a gradual reduction in chromosome number from the diploid number of 40 to the haploid complement; at higher doses, embryos did not proceed beyond the first cleavage division (Edwards, 1954). In the Chinese hamster, mouse, and man, the "X_2" and Y chromosomes can be lost completely without accompanying lethality (Yerganian, 1963; Yerganian, et al., 1964).

The fertility of the offspring of X-irradiated males has been studied as an index of radiation damage. The rate of induction of dominant sterility in the male offspring of presterile matings is far from negligible in the mouse and has been estimated at 10 percent for an exposure of 700 r (Russell, 1954). The mutational changes involved in the production of dominant sterility are not known although it is possible that sterility in some cases might be caused by the induction of multiple translocations giving rise to aneuploid gametes (Russell, 1954). Evidence for the role of reciprocal translocations in dominant sterility has been observed: 571 offspring of X-irradiated male mice were examined; in seven males in which spermatozoa were rare or absent, one or more translocation figures were found in the spermatocytes. Two mice with histological abnormalities had no evidence of visible chromosomal rearrangements; four animals did not have spermatocytes and thus could not be

tested for translocations (Leonard and Deknudt, 1968). Much more attention has been given to the induction of semi-sterility (Russell, 1954).

Snell examined the litters of the crosses of F_1 offspring of X-irradiated male mice (600 r) with normal females and found a bimodal distribution of litter size with one mode corresponding to control values and the other mode corresponding to smaller litter size. He determined that one-fourth of the F_1 produced small litters and thus called them semi-steriles (Snell, 1934; Snell, 1935). His genetic evidence indicated that the semi-sterility was due to reciprocal translocations; first, semi-sterility was transmitted as a dominant to approximately half (43.9 percent) of the surviving progeny of outcrosses of the semi-steriles with normal animals; secondly, in one translocation involving chromosomes 5 and 8 the normally unlinked genes "a" and "b" were both linked with semi-sterility (Snell, 1934, 1935, 1946). Koller and Auerbach (1941) were the first investigators to observe a translocation cytologically in a semi-sterile mouse; in a meiotic preparation they found a ring or chain-of-four configuration. Russell studied extensively the frequency of semi-sterility in mice and found a frequency of 25 percent in the F_1 for exposures of 500, 750, and 1000 r; however, most of the semi-steriles were not examined cytologically or genetically for the presence of translocations. Russell assumed that semi-sterility was a rough estimate of reciprocal translocation induction (Russell, 1954).

Hertwig (1938) and Russell (1954) both reported that offspring conceived during the lasting period of fertility seldom exhibited semi-sterility; however, a later investigation by Griffen proved that translocations were induced in the spermatogonia of irradiated mice as evidenced

by the appearance of semi-sterility in offspring of poststerile matings. Cytological proof of a translocation was found in a meiotic preparation from a male offspring of a semi-sterile (Griffen, 1958). These findings have been confirmed by Leonard and Deknudt (1968) and by Lyon and Smith (1971). Translocations induced in mammalian spermatogonia are studied extensively now because of the ease of their detection in the diakinesis or first metaphase stage of spermatocytes (Leonard and Deknudt, 1968; Lyon and Smith, 1971).

As can be seen from the preceding discussion, the genetic effects of acute, paternal X-irradiation in mammals have been extensively studied, and, as in the case of the reciprocal translocation, a cytological basis (chromosomal aberration) has been observed for the genetic effect. Since the karyotypes of the mammals used in the radiogenetic work are not favorable for cytological analysis, it would be informative to examine embryos and offspring for the presence of cytologically observable chromosomal aberrations in a species with a favorable karyotype. Thus the Chinese hamster was chosen for this study.

The Chinese hamster, Cricetulus griseus Milne-Edw., a nocturnal rodent native to China, was selected for this particular study because it is unique among Eutherian laboratory mammals in that it has a small number of chromosomes of which all but four small pairs are morphologically distinct (Yerganian, 1958, 1959a; Ford and Yerganian, 1958). The chromosomal complement consists of 22 chromosomes--10 pairs of autosomes and one pair of sex chromosomes (Yerganian, 1959b), as shown in Figures 1-4. The autosomes are divided into four groups based on their appearance in the c-metaphase cell as follows: (1) pairs 1 and 2; (2) pairs 4 and 5; (3) pairs

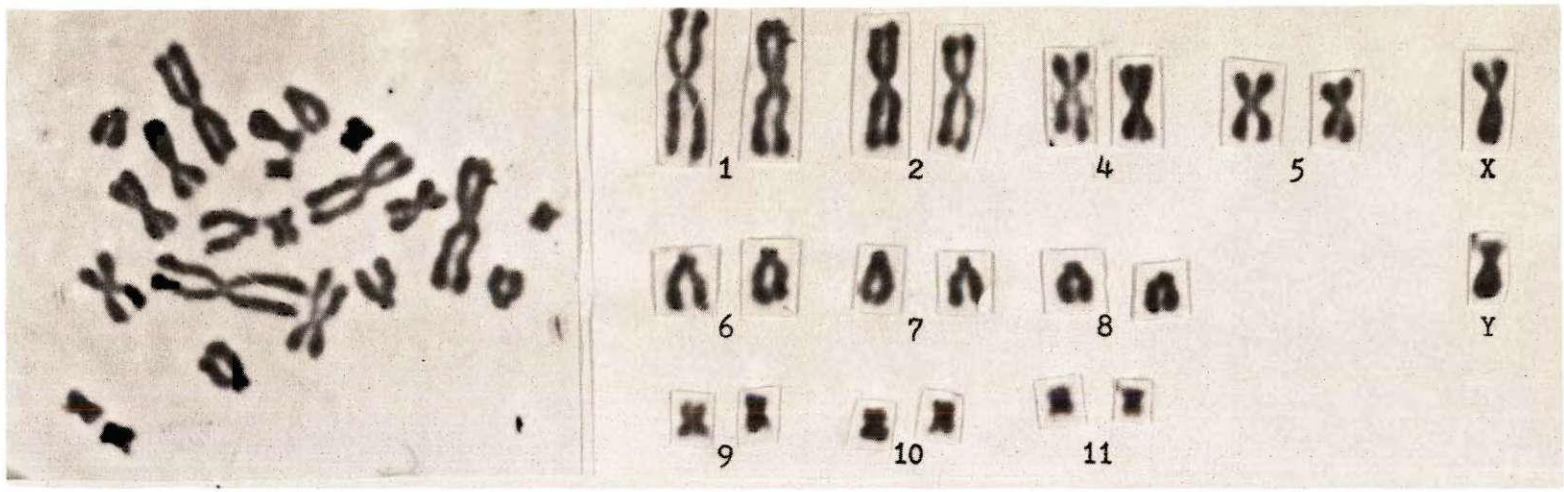

Figure 1. Karyotype of Cricetulus griseus Milne-Edw. From Embryonic Cells Cultured in Vitro (X7,000).

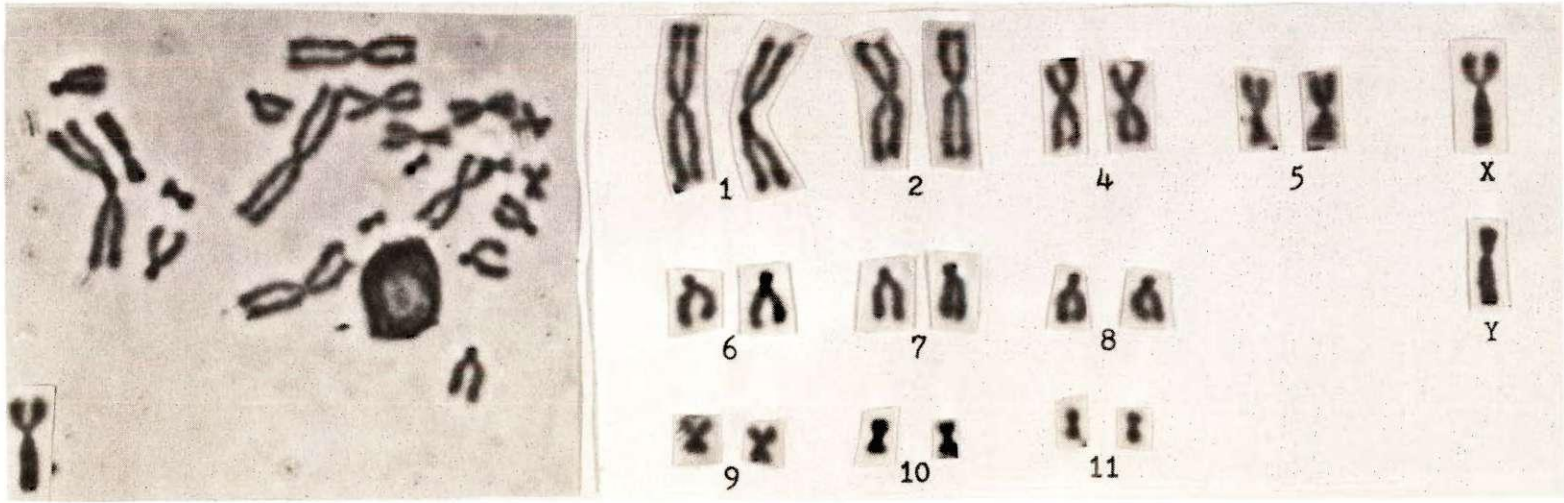

Figure 2. Karyotype of Cricetulus griseus Milne-Edw. From Embryonic Cells Cultured in Vitro (X7,000).

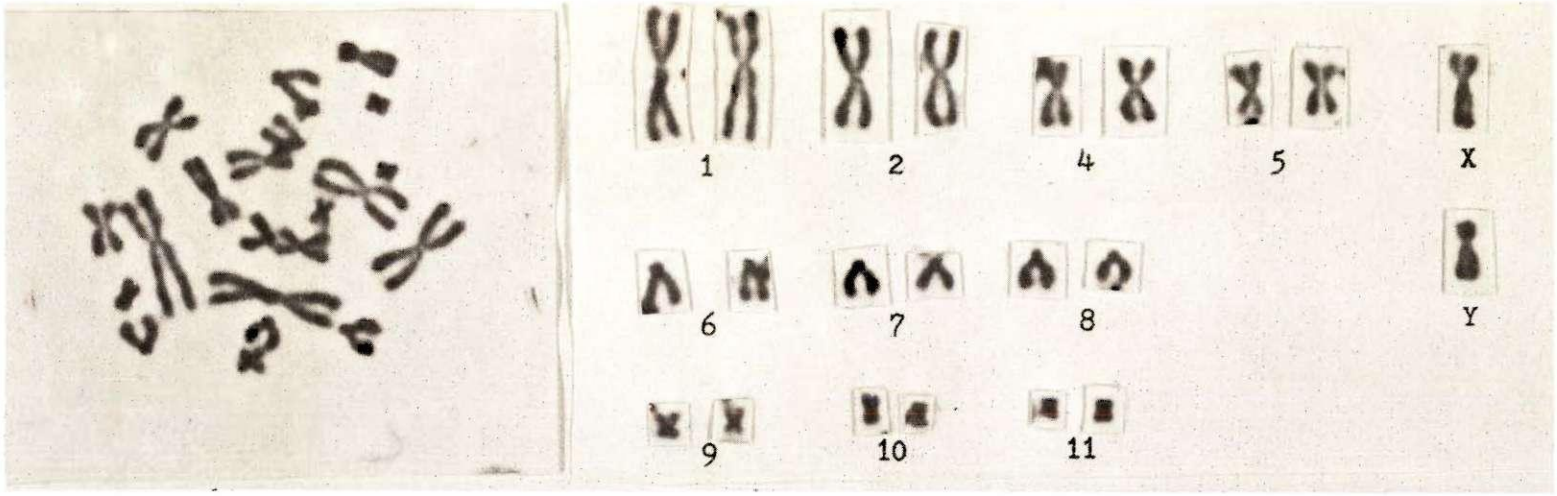

Figure 3. Karyotype of Cricetulus griseus Milne-Edw. From Embryonic Cells Cultured in Vitro (X7,000).

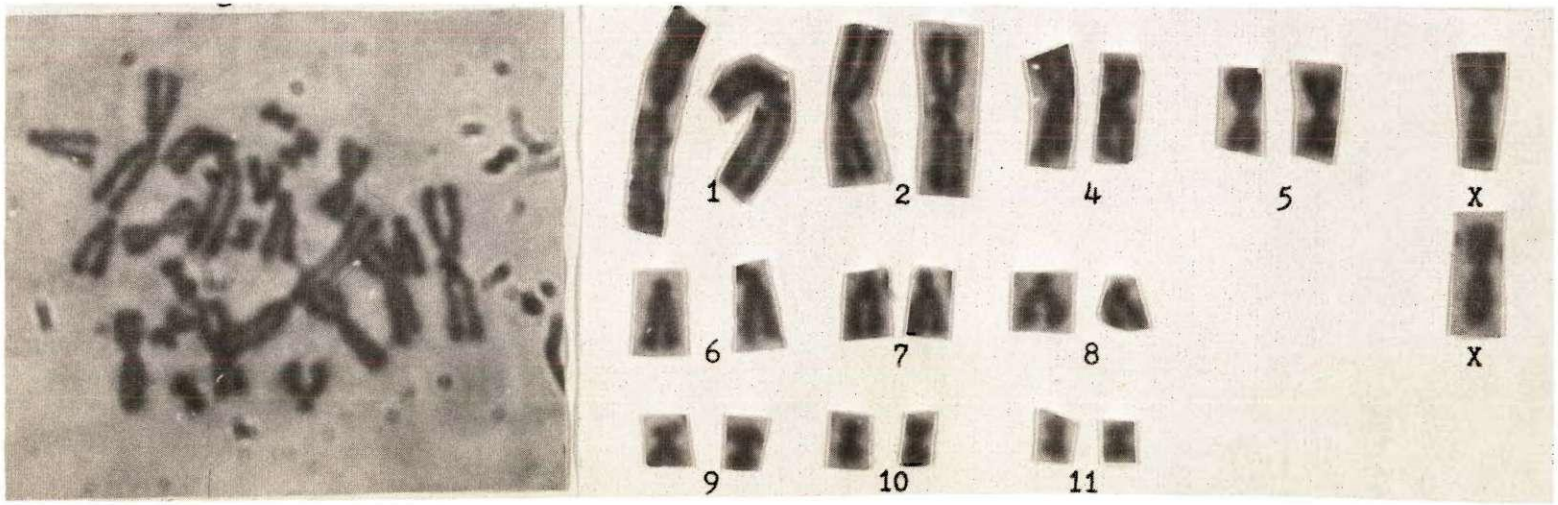

Figure 4. Karyotype of Cricetulus griseus Milne-Edw. From Embryonic Cells Cultured in Vitro (X7,000).

9, 10, 11; and (4) pairs 6, 7, 8. Groups (1), (2), and (3) consist of metacentric chromosomes; group (4) consists of chromosomes with subterminal centromeres (Yerganian, 1959a; Fredga and Santesson, 1964). It is difficult to distinguish pair 7 from pair 8 and pair 10 from pair 11. The X chromosome is slightly larger than the chromosomes of pair 4; the Y chromosome is smaller than the chromosomes of pairs 4 and 5 and has a submedian centromere (Yerganian, 1959b; Fredga and Santesson, 1964). The X chromosome of the normal Chinese hamster female has been found to have two forms--the X_1 and the X_2 (Yerganian, 1960, 1963). The X_1 chromosome, also found in the male, has an euchromatic, heavily-spiraled short arm and a slender, heterochromatic long arm which has a secondary constriction (this constriction may be unnoticed in colchicine pre-treated cells because colchicine increases spiralization) (Yerganian, 1960, 1963). The X_2 chromosome, found only in the female, is totally heterochromatic and has no secondary constriction (Yerganian, 1963).

Identification of the chromosome pairs is facilitated by the use of three diagnostic characteristics defined as follows: (1) the length of each chromosome relative to the total length of a normal, X-containing haploid set; (2) the arm ratio of the chromosomes expressed as the length of the longer arm divided by that of the shorter arm; and (3) the centromere index expressed as the ratio of the length of the shorter arm to the whole length of the chromosome (Ford and Yerganian, 1958; Robinson, 1961; Yerganian, 1963). The centromere index is used in this study.

Only a few radiation parameters have been determined for the Chinese hamster. The $LD_{50(40)}$ for the male Chinese hamster has been found

to be 856 rads, and the $LD_{50(100)}$ has been found to be 846 rads. The degree of inbreeding has not been found to alter the LD_{50} significantly (Corbascio, Kohn, and Yerganian, 1962). Bender and Gooch (1961) found that the in vivo breakage frequency six hours after irradiation in bone marrow cells was 0.0021 breaks per cell per r. The frequencies of chromosome breaks in bone marrow cells and spermatogonia of the Chinese hamster six hours after irradiation have been determined, respectively, as 0.015 + 0.0015 r* and 0.032 + 0.0041 r breaks per cell (Brooks and Lengemann, 1967). Fetner (1970) found a frequency of 0.0086 + 0.00194 R** chromosome exchanges per cell in primary cell culture 20 hours after irradiation. Chinese hamster cells irradiated in vitro and in vivo and sampled shortly thereafter exhibit all the expected aberration types (Bender and Gooch, 1961; Fetner, 1970). The frequency of breaks in spermatozoa is not known and can only be inferred from damage manifest after fertilization.

The existence and time course of a fertile-sterile-fertile sequence for the Chinese hamster has not been established as yet. All matings in our study took place within ten days after X-irradiation; we assume that we are studying the effect of radiation-induced damage during the presterile period in the male (i.e., on spermatozoa, spermatids, and secondary spermatocytes). Nucleic acid labeling studies indicate that 11 days elapse between the labeling of secondary spermatocytes and spermatids and the labeling of spermatozoa (Utakoji, 1966). Radiation has been shown not to

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*r = exposure in roentgens

**R = dose in rads

affect the time sequence of spermiogenesis in the mouse (Hertwig, 1938; Eschenbrenner and Miller, 1950; Russell and Saylors, 1963).

Since this study utilizes cell culture as a means of producing sufficient cells for study, it is necessary to consider the effects of cell culture on chromosome morphology. Although the in vivo spontaneous frequency of aberrations in the bone marrow of the Chinese hamster is essentially zero (Bender and Gooch, 1961), the frequency of spontaneous aberrations increases in a primary cell culture as the age of the culture increases (Hsu, 1962). Ford and Yerganian (1958) and Hatfield (1968) confirm that the karyotype begins to undergo changes immediately after in vitro culture; however, at 33 days after the initiation of the culture, 84 percent of the cells studied by Ford and Yerganian were still diploid. No preparation examined in this study was grown in culture for more than 28 days.

CHAPTER II

MATERIALS AND METHODS

Source, Breeding, and Maintenance of the Animal Colony

The animal chosen for study is the striped-back or Chinese hamster, Cricetulus griseus Milne-Edw., a nocturnal rodent which has unique advantages for cytogenetic research because it possesses the lowest number of chromosomes yet observed among Eutherian laboratory mammals and because all but four of the small pairs of the eleven pairs of chromosomes are morphologically distinct (Yerganian, 1958).

An inbred colony, which originated from animals obtained from Dr. George Yerganian of the Children's Cancer Research Foundation in Boston, is maintained at the Georgia Institute of Technology by Dr. R. H. Fetner. An artificial lighting system is employed in the animal quarters so that the animals are in artificial light from 6:00 a.m. to 8:00 p.m. and in complete darkness from 8:00 p.m. to 6:00 a.m. Temperature in the animal quarters is maintained at approximately room temperature (21°-25°C). The colony is propagated by a system of brother-sister matings since litters raised together show a greater tendency to mate and a lower tendency to mutilate one another (Yerganian, 1958).

For breeding purposes a single female was placed in a large breeding cage with her male litter-mates for ten days, a period chosen to give a large percentage of pregnancies since the estrous cycle of this hamster

is approximately four days (Yerganian, 1958). The gestation period as determined by Yerganian (1958) is twenty days.

X-irradiation

Male hamsters (5-12 months in age) were X-irradiated. A constant potential Westinghouse X-ray generator operated at 250 KV, 15 ma was used for whole-body irradiation. Copper (0.5 mm) and aluminum (1.0 mm) filters produced radiation equivalent to a half-value layer of 1.55 mm copper. Radiation exposure was measured with a Victoreen 250 roentgen intermediate energy chamber at a target-subject distance of 48 cm, and was found to be approximately 80 roentgens (r) per minute. Male hamsters to be irradiated were placed at the same target-subject distance in a large beaker centered under the beam. A total whole-body exposure of 400 roentgens was given.

Female litter mates were mated with males immediately after irradiation. The females were divided into two groups as follows: one group was sacrificed 15 days after X-irradiation and the excised embryos cultured *in vitro*; the other group was permitted to deliver their offspring. Lung cultures were later prepared from these offspring. Control groups were treated in the same manner except that the males were not irradiated. The subsequent experimental procedures will be described in detail in the following sections.

Source and Growth of Cells

A. Embryo Culture

Gravid females were anesthetized with carbon dioxide and sacrificed by cervical dislocation five days after they had been removed from their

male litter mates. The embryos and surrounding uterine tissue were removed by aseptic surgery and placed in small sterile plastic dishes containing culture media. The placenta and embryonic membranes were removed and discarded. The embryos were placed in separate dishes where they were finely minced with surgical scissors while observing aseptic technique. The minced tissue was transferred to sterile 250 ml plastic tissue culture flasks (Falcon Company, Los Angeles, California) to which 20 to 30 ml culture media (see Table 8, Appendix) was added. Growth proceeded as described in Section C.

B. Lung Culture

Young hamsters, approximately one month old, were sacrificed as in Section A. The lungs were removed observing aseptic technique and rinsed in culture media in a small sterile plastic dish to remove excess blood. The lung tissue was then transferred to another sterile plastic dish where it was finely minced. The minced tissue was transferred to sterile 250 ml plastic tissue culture flasks and culture media was added.

C. Growth of Cells

The culture flasks were placed in an incubator maintained at 35°C. Media was replaced on the first and second days and every second day thereafter. Cultures were periodically examined using an inverted microscope. When a uniform cell sheath covered the bottom of the flask, a silicone policeman was used to scrape the cells from the surface, the flask was agitated to suspend the cells, and part of the fluid was transferred to large mouth French square bottles in which a sterile glass microscope slide had been placed. When a uniform cell sheath had grown on the slide,

the slide was prepared for cytological observation as will be described in the following section.

Slide Preparation

Slides were prepared for examination of chromosomes when a uniform cell sheath covered the slide. To arrest cell division, enough of a stock solution of colchicine was added to the culture bottles to yield a final concentration of two milligrams per liter, and the bottles were returned to the incubator. After five to six hours, the media in the bottle was made hypotonic to the cells by the addition of sufficient distilled water at 35°C to dilute the culture media to 20 percent (v/v). After 20 minutes in the hypotonic media, the slides were immersed for 20 minutes in a Coplin jar containing a modified Carnoy's fixative composed of 95 percent ethanol (three parts) and glacial acetic acid (one part). The Coplin jar containing the slides was then placed in a dry ice freezer for 10 to 15 minutes to prevent heat distortion in the subsequent flaming. The slides were then removed from the freezer, excess fluid was drained off, and the slides were flamed to flatten the cells.

All observations were made with stained material. When stained slides were needed, an orcein stain was prepared fresh for each day's use. To prepare the stain, a 50 percent (w/v) aqueous solution of propionic acid was saturated with orcein, boiled gently, cooled, and filtered. An acetic orcein stain was also used; 0.5 gm of orcein was added to 11 ml of boiling glacial acetic acid; the solution was cooled, 14 ml water was added, and the solution was filtered.

In staining, a few drops of either of the stains were placed on

the slide, a cover slip added, excess stain blotted away, and the cover slip was sealed by applying molten printer's wax to its edges. This procedure is modified from that of Ford and Hamerton (1956).

Analysis

A Leitz (Wetzlar) phase contrast microscope was used to examine the stained slides. The 100X oil immersion objective was used with a green filter for all photomicrographs resulting in a magnification of 800. Only those metaphase cells which were relatively flat, which exhibited minimum chromosome overlap, and in which the nuclear envelope was intact were used for analysis.

Thirty cells per slide were analyzed for chromosome number and obvious aberrations (i.e., rings, dicentrics, reciprocal translocations, large deletions). Five cells per slide were photographed for subsequent detailed analysis. An Exakta 35 mm camera with Panatomic X film was used. Several enlarged (4 x 5) prints of each photograph were made on Kodabromide F-4 double-weight paper and developed with standard procedure in Kodak Dektol.

Individual chromosomes were cut from these prints. The chromosome pairs were then matched and taped with transparent tape to graph paper. To determine the presence of any subtle defects, measurements of the chromosomes were made in the following manner. Twenty-two gauge solder wire was placed on one arm of the chromosome and bent to the shape of the chromosome arm. Marks were made on the wire with a Rapidograph pen to locate both ends of the chromosome arm and the centromere. The wire was then straightened and measured against a ruler graduated in 0.5 millimeters.

Each chromosome arm was measured twice in this manner. The centromere index (the length of the short arm divided by the entire length of the chromosome) and the homologue ratio (the total length of the longer member of the chromosome pair divided by the total length of the shorter homologue) were calculated. The centromere index was calculated for each member of the chromosome pair. Means and standard errors were calculated.

CHAPTER III

RESULTS

Eleven males were irradiated and mated for the purpose of obtaining embryos for culture. Four matings did not result in pregnancy. In five of the seven pregnancies there were abnormalities; there were large uterine swellings which did not contain any visible embryonic tissue. These swellings were deciduous tissue, or the maternal portion of the placenta (Turner, 1966), and were probably sites of resorption of defective embryos. Such swellings may also be the manifestation of pseudopregnancy which can be caused by mating with a sterile male as well as by other means (Turner, 1966); however, pseudopregnancy does not result in any visible changes in the uterine lining in the golden hamster (Kenter, 1968). In animals in which embryos and sites of resorption were present, the sites of resorption were smaller in size than the embryonic vesicles. No similar swellings or sites of resorption were observed in control pregnancies. Table 1 shows the frequencies of embryos and sites of resorption in the seven pregnant hamsters.

It can be seen from Table 1 that 52 percent of the embryos were reabsorbed for an exposure of 400 r. If all the embryos had survived to birth the average litter size would have been 2.4 ± 0.4 (the animals in which there were no embryos would not be included in the calculation since they would fail to produce any young at term). Fetner (unpublished data) found that an exposure of 400 r to males resulted in a litter size of

2.68 ± 0.24; control litter size was found to be 5.00 ± 0.38.

Table 1. Numbers of Sites of Resorption and Number of Embryos in Females Impregnated by Males Receiving 400 r

Animal	Number of Sites of Resorption	Number of Embryos	Percent Resorption Observed	Total Implantations
1	1	0	100	1
2	4	0	100	4
3	5	2	71	7
4	2	3	40	5
5	1	1	50	2
6	0	3	0	3
7	0	3	0	3
Totals	13	12	52*	25

*Average value.

Nine of the embryos cultured grew well enough for analysis. An attempt was made to culture some of the interior mass of the sites of resorption, but none of these cultures grew.

Offspring of seven irradiated males were sacrificed at one month of age for the purpose of obtaining lung cultures. The mean litter size at one month was 3.1 ± 0.7. The lung tissue did not grow as well or as quickly in culture as did the embryonic tissue. Seven cultures grew sufficiently well for analysis.

No aberrations were observed in the controls. No ring or dicentric chromosomes were observed in any of the experimental animals sired by X-irradiated males.

Two reciprocal translocations and a monosomic chromosome were found in the animals sired by X-irradiated males. The karyotype of one cultured

embryo contained a chromosomal aberration, a reciprocal translocation. The reciprocal translocation was present in all 30 cells examined; the exchange took place between chromosome 1 and chromosome 6. The karyotype is shown in Figures 5-8. The long arm of aberrant chromosome 1 corresponded to the long arm of the normal chromosome 1; the interchange involved the short arm of chromosome 1 and the long arm of chromosome 6. The total length of the two chromosomes involved in the translocation was 101 percent of the length of the normal homologues. The embryo appeared normal to gross observation.

A reciprocal translocation was also found in an experimental lung culture (lung culture 1 in Tables 2-5). This translocation involved a transfer between the short arm of chromosome 1 and the long arm of chromosome 7; in addition, a monosomic chromosome was present: one chromosome from either pair 4 or the sex chromosome pair was missing. These characteristics were observed in all 30 cells examined. The karyotype is shown in Figures 9-12. Measurements made on 30 cells showed that 98 percent of the total length of the normal chromosomes was represented in the two chromosomes involved in the reciprocal translocation. Although the animal appeared to be a normal female, it was difficult to distinguish between chromosome 4 and one of the chromosomes designated as an X chromosome. It is possible that this hamster was an XO individual.

Measurements were made on chromosome pairs 1 and 2 in order to detect subtle changes in the morphology of the chromosomes. Measurements were made on five cells (30 cells for controls--five cells from each of six animals) unless otherwise indicated. The centromere index (the length of the short arm divided by the entire length of the chromosome) was

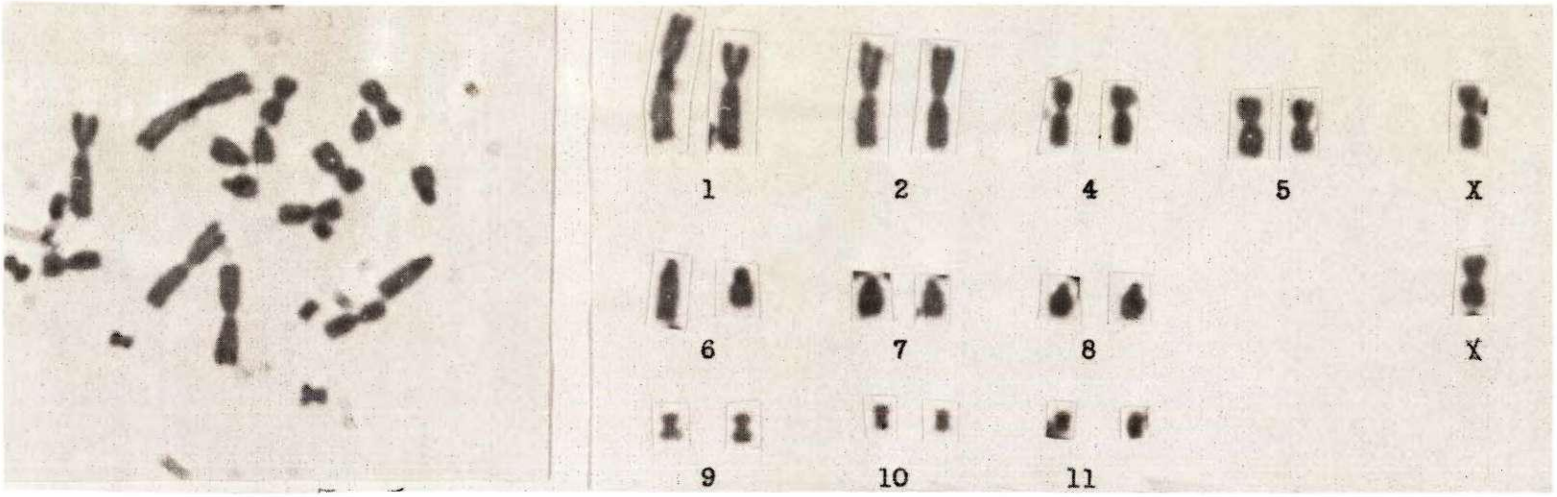

Figure 5. Abnormal Karyotype Showing Reciprocal Translocation. From in Vitro Culture of Cells of Embryo Sired by Irradiated Male Cricetulus griseus (X6,000).

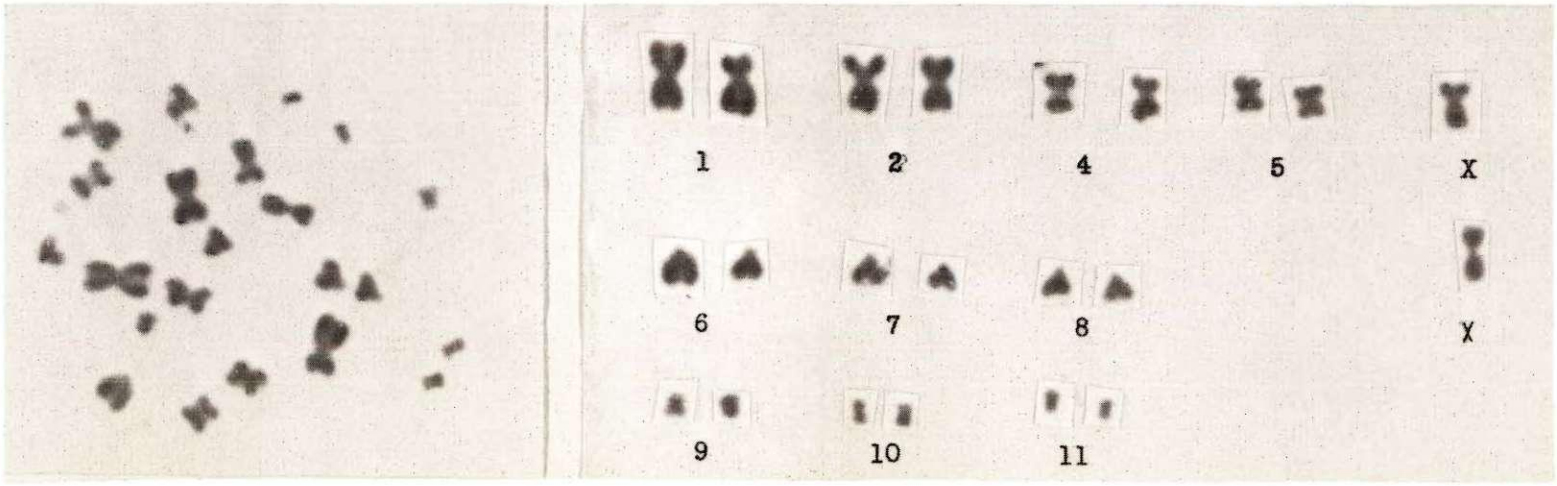

Figure 6. Abnormal Karyotype Showing Reciprocal Translocation. From in Vitro Culture of Cells of Embryo Sired by Irradiated Male Cricetulus griseus (X6,000).

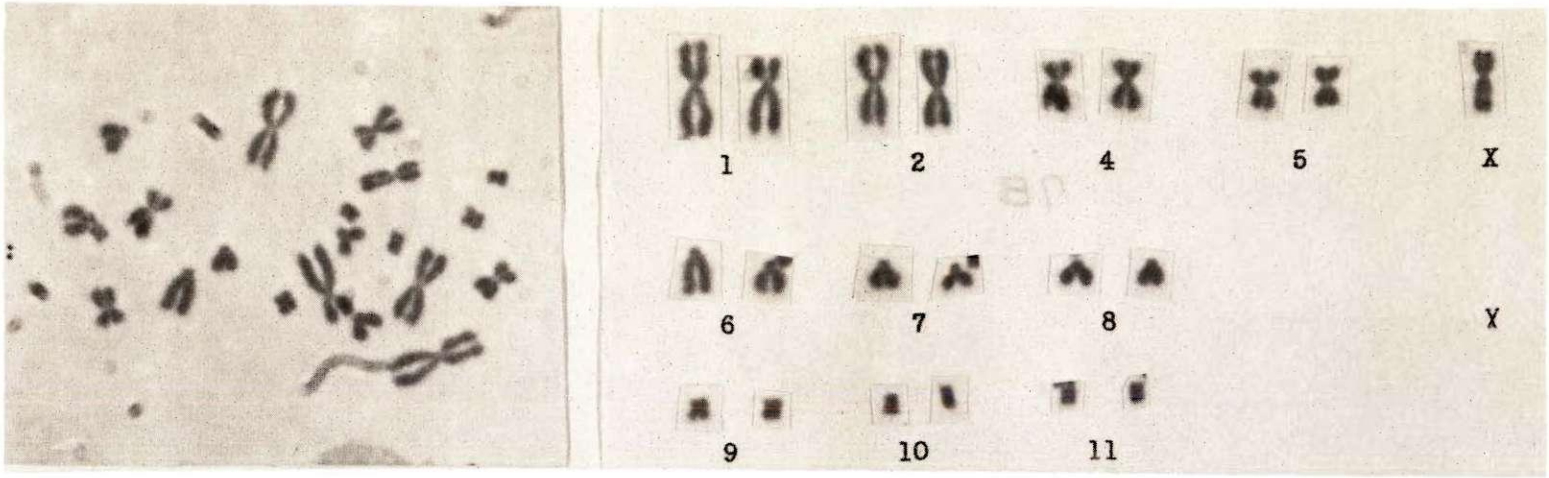

Figure 7. Abnormal Karyotype Showing Reciprocal Translocation. From in Vitro Culture of Cells of Embryo Sired by Irradiated Male Cricetulus griseus (X6,000).

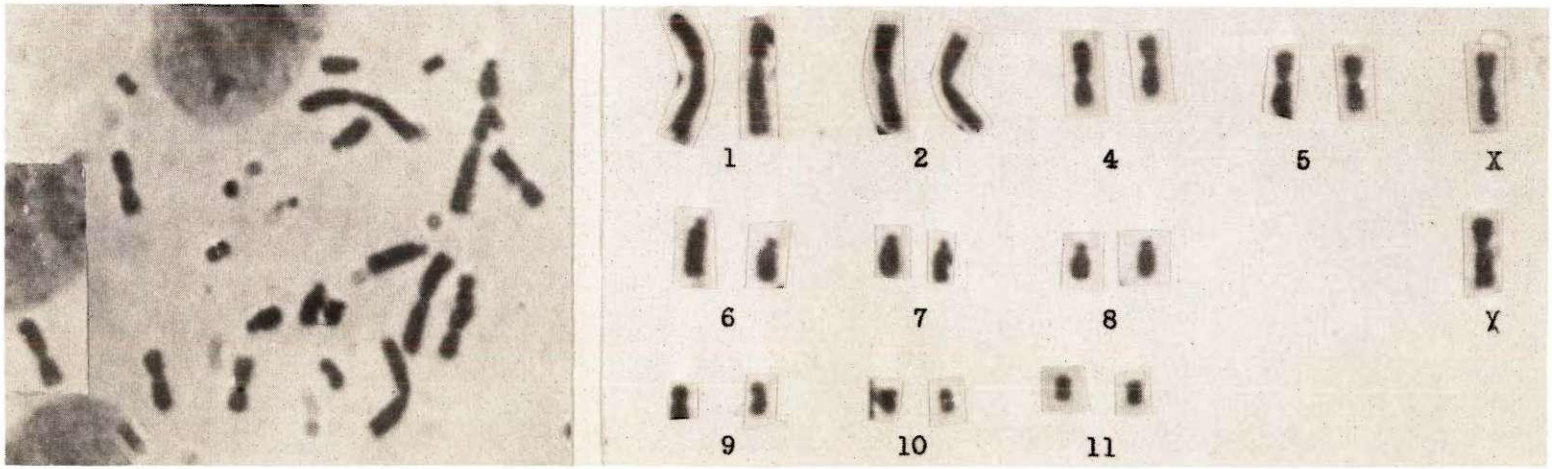

Figure 8. Abnormal Karyotype Showing Reciprocal Translocation. From in Vitro Culture of Cells of Embryo Sired by Irradiated Male Cricetulus griseus (X6,000).

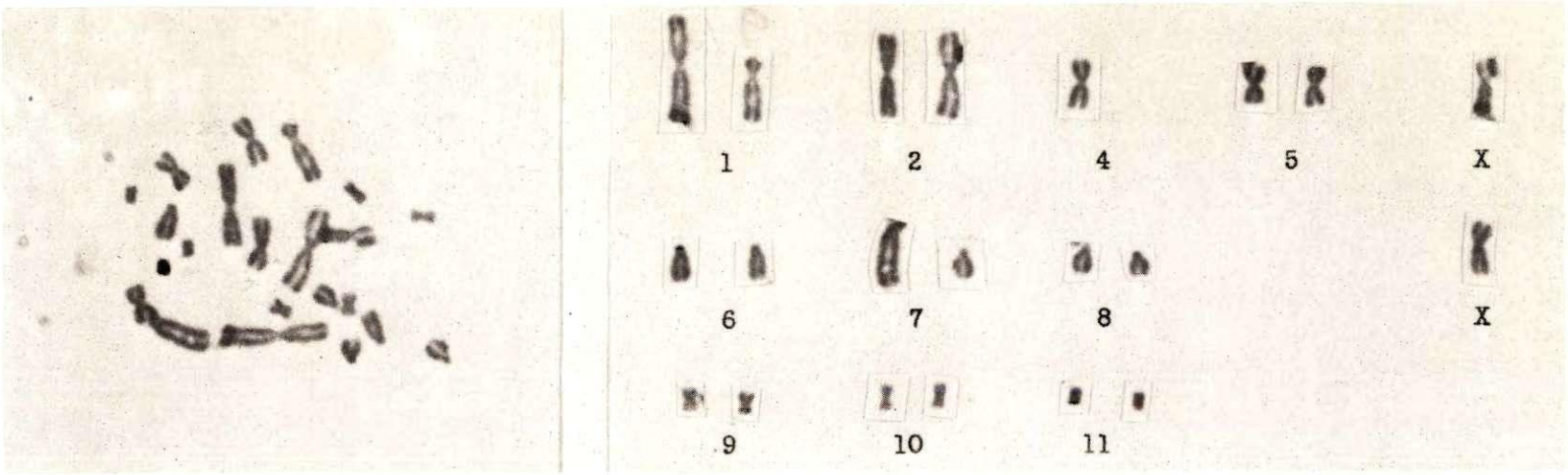

Figure 9. Abnormal Karyotype Showing Reciprocal Translocation. From Lung Tissue, Cultured in Vitro, of Offspring of Irradiated Male Cricetulus griseus (X6,000).

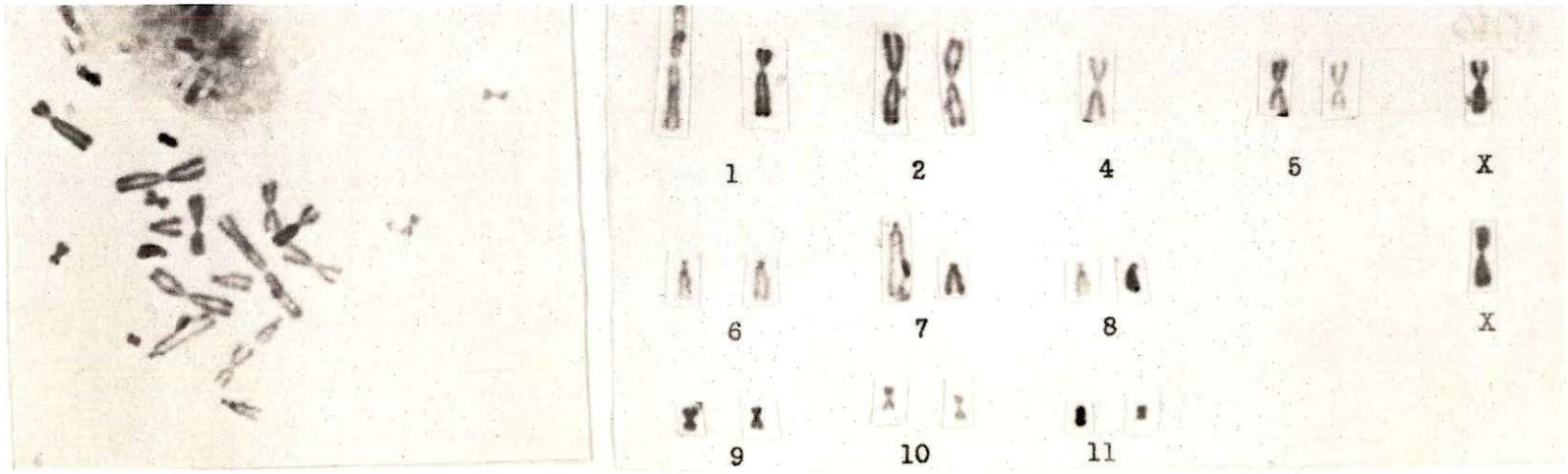

Figure 10. Abnormal Karyotype Showing Reciprocal Translocation. From Lung Tissue, Cultured in Vitro, of Offspring of Irradiated Male Cricetulus griseus (X6,000).

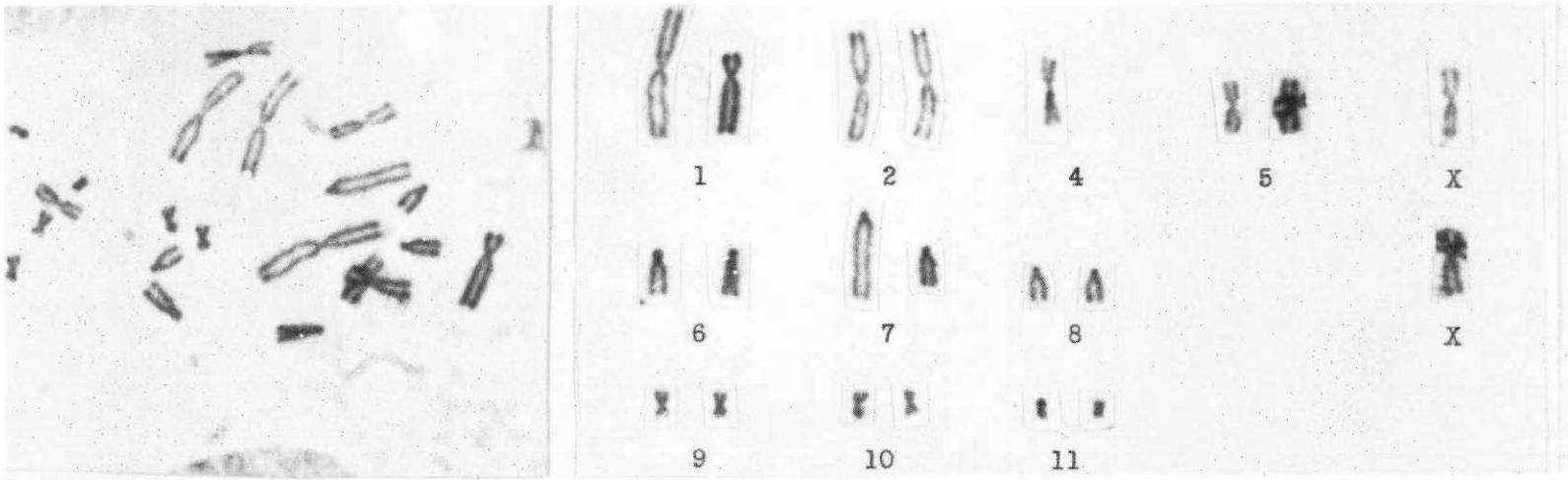

Figure 11. Abnormal Karyotype Showing Reciprocal Translocation. From Lung Tissue, Cultured in Vitro, of Offspring of Irradiated Male Cricetulus griseus (X6,000).

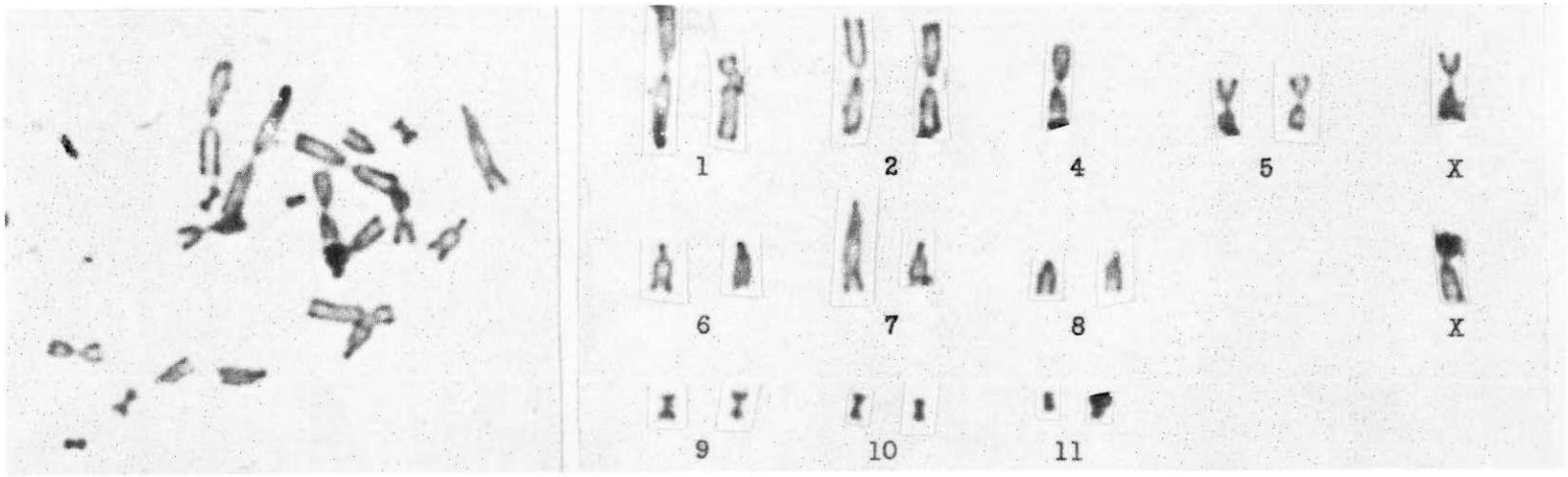

Figure 12. Abnormal Karyotype Showing Reciprocal Translocation. From Lung Tissue, Cultured in Vitro, of Offspring of Irradiated Male Cricetulus griseus (X6,000).

calculated for both members of the homologue pair; the homologue ratio (the total length of the longer member of the chromosome pair divided by the total length of the shorter homologue) was also calculated. Means and standard errors were calculated.

In the cells analyzed one haploid set of chromosomes is derived from the X-irradiated father and one haploid set of chromosomes is derived from the non-irradiated mother; i.e., each chromosome pair consists of an irradiated and a non-irradiated chromosome. All of the cells should have the same aberration, if any, since these cells are all derived from a single male gamete; only if a breakage-fusion-breakage cycle took place would the aberration be different in different cells. The centromere index would be affected by such aberration types as interstitial and terminal deletions, pericentric inversions, rings (inter-arm deletions), dicentrics (deletions), and reciprocal translocations between arms of one chromosome. The homologue ratio would not be affected by pericentric inversions and reciprocal translocations between the arms of one chromosome.

For the centromere index, means were not compared because calculation of the mean from the experimental would include both irradiated and non-irradiated chromosomes and would thus underestimate the damage when compared to controls. Variances were compared by means of an F-test. For the homologue ratio, an F-test could not be used since forming a ratio eliminates the X-ray induced source of variation; except for experimental error, this ratio should be the same from cell to cell, because all cells should have the same aberration. Thus a t-test was used to compare the homologue ratio to that of the controls although it would underestimate

the effects of irradiation. Statistical formulae and methods are presented in the statistical appendix.

For chromosome pair 1 the centromere index (Table 2) was found to be 0.445 ± 0.003 for control embryos and 0.447 ± 0.004 for control lung cultures. A significant difference was found for 22 percent of the experimental embryos and for 29 percent of the experimental lung cultures. The centromere index for chromosome pair 2 (Table 3) was found to be 0.471 ± 0.008 for control embryos and 0.468 ± 0.002 for control lung cultures. There were significant differences for 44 percent of the experimental embryos and 43 percent of the experimental lung cultures.

The homologue ratio for chromosome pair 1 (Table 4) was calculated as 1.06 ± 0.01 for control embryo cultures and 1.06 ± 0.01 for control lung cultures. Eleven percent of the experimental embryos showed a significant difference in the homologue ratio and 29 percent of the experimental lung cultures showed significant differences. The homologue ratio for chromosome pair 2 (Table 5) was calculated as 1.06 ± 0.01 for control embryos and 1.04 ± 0.01 for control lung cultures. Eleven percent of the experimental embryos exhibited a significant difference, and 14 percent of the experimental lungs differed significantly.

Two of the experimental lung cultures came from animals that were considerably smaller in size than the rest of the litter; since Yerganian (1963) thought that such animals might bear chromosomal abnormalities, it was of interest to note whether these animals' karyotypes differed from controls. No gross aberrations were observed. Statistical analysis showed no significant difference in chromosome pairs 1 and 2 for the male runt (lung culture 5). For the female runt (lung culture 6) there was a significant difference in the homologue ratio for chromosome pair 1.

Table 2. The Centromere Index Calculated for Chromosome Pair 1 and Results of F-test

	Number of Cells Analyzed[a]	Centromere Index ± Standard Error	Value of F	Significance[b]
Embryonic Tissue Culture				
Control	30	0.445 ± 0.003	----	----
Experimental				
1	5	0.437 ± 0.010	1.59	N. S.
2	5	0.451 ± 0.008	1.30	N. S.
3	5	0.443 ± 0.007	1.21	N. S.
4	5	0.456 ± 0.008	1.02	N. S.
5	5	0.426 ± 0.015	4.05	S. (0.05, 0.01)
6	4	0.448 ± 0.008	1.14	N. S.
7	5	0.435 ± 0.010	1.68	N. S.
8	13	0.399 ± 0.013	7.59	S. (0.05, 0.01)
9	16	0.438 ± 0.005	1.21	N. S.
Lung Tissue Culture				
Control	30	0.447 ± 0.004	----	----
Experimental				
1	5	0.380 ± 0.025	6.75	S. (0.05, 0.01)
2	5	0.440 ± 0.007	1.99	N. S.
3	5	0.438 ± 0.008	1.37	N. S.
4	6	0.423 ± 0.017	3.92	S. (0.05, 0.01)
5	5	0.455 ± 0.009	1.19	N. S.
6	5	0.450 ± 0.007	1.59	N. S.
7	5	0.449 ± 0.009	1.08	N. S.

[a]For centromere index, N(sample size) is twice the number of cells analyzed because the centromere index was computed for each member of the chromosome pair.

[b]N. S. means that there is no significant difference between experimentals and controls. S. (0.05, 0.01) means a significant difference at probability = 0.05 and at probability = 0.01.

Table 3. The Centromere Index Calculated for Chromosome Pair 2 and Results of F-test

	Number of Cells Analyzed[a]	Centromere Index ± Standard Error	Value of F	Significance[b]
Embryonic Tissue Culture				
Control	30	0.471 ± 0.008	----	----
Experimental				
1	5	0.463 ± 0.006	1.17	N. S.
2	5	0.452 ± 0.009	2.35	S. (0.05)
3	5	0.446 ± 0.013	4.65	S. (0.05, 0.01)
4	5	0.454 ± 0.007	1.13	N. S.
5	5	0.464 ± 0.004	2.43	N. S.
6	4	0.460 ± 0.011	2.42	S. (0.05)
7	5	0.469 ± 0.005	1.41	N. S.
8	6	0.446 ± 0.013	5.32	S. (0.05, 0.01)
9	16	0.467 ± 0.006	1.52	N. S.
Lung Tissue Culture				
Control	30	0.468 ± 0.002	----	----
Experimental				
1	5	0.446 ± 0.012	4.38	S. (0.05, 0.01)
2	5	0.471 ± 0.006	1.19	N. S.
3	5	0.468 ± 0.005	1.54	N. S.
4	6	0.453 ± 0.009	2.59	S. (0.05, 0.01)
5	5	0.460 ± 0.005	1.18	N. S.
6	5	0.463 ± 0.008	1.88	N. S.
7	5	0.447 ± 0.014	5.35	S. (0.05, 0.01)

[a]For centromere index, N(sample size) is twice the number of cells analyzed because the centromere index was computed for each member of the chromosome pair.

[b]N. S. means that there is no significant difference between experimentals and controls. S. (0.05) means there is a significant difference at probability = 0.05. S. (0.05, 0.01) means there is a significant difference at probability = 0.05 and at probability = 0.01.

Table 4. The Homologue Ratio Calculated for Chromosome Pair 1 and Results of T-test

	Number of Cells Analyzed	Length Ratio ± Standard Error	Value of t	Significance[a]
Embryonic Tissue Culture				
Control	30	1.06 ± 0.01	----	----
Experimental				
1	5	1.05 ± 0.01	1.00	N. S.
2	5	1.10 ± 0.04	1.14	N. S.
3	5	1.08 ± 0.02	1.33	N. S.
4	5	1.05 ± 0.02	0.42	N. S.
5	5	1.06 ± 0.02	0	N. S.
6	4	1.07 ± 0.01	1.25	N. S.
7	5	1.05 ± 0.01	0.71	N. S.
8	13	1.17 ± 0.02	6.88	S. (0.05, 0.01)
9	16	1.05 ± 0.02	0.59	N. S.
Lung Tissue Culture				
Control	30	1.06 ± 0.01	----	----
Experimental				
1	5	1.58 ± 0.03	18.57	S. (0.05, 0.01)
2	5	1.05 ± 0.01	0.77	N. S.
3	5	1.07 ± 0.02	0.56	N. S.
4	6	1.09 ± 0.04	0.79	N. S.
5	5	1.06 ± 0.02	0	N. S.
6	5	1.03 ± 0.01	3.00	S. (0.05)
7	5	1.07 ± 0.02	0.48	N. S.

[a] N. S. means that there is not a significant difference between experimentals and controls. S. (0.05) means that there is a significant difference at probability = 0.05. S. (0.05, 0.01) means that there is a significant difference at probability = 0.05 and at probability = 0.01.

Table 5. The Homologue Ratio Calculated for Chromosome Pair 2 and Results of T-test

	Number of Cells Analyzed	Length Ratio ± Standard Error	Value of t	Significance[a]
Embryonic Tissue Culture				
Control	30	1.06 ± 0.01	----	----
Experimental				
1	5	1.08 ± 0.02	0.83	N. S.
2	5	1.10 ± 0.05	0.51	N. S.
3	5	1.14 ± 0.04	2.22	N. S.
4	5	1.08 ± 0.02	1.18	N. S.
5	5	1.02 ± 0.01	4.44	S. (0.05)
6	4	1.09 ± 0.04	0.86	N. S.
7	5	1.10 ± 0.03	1.18	N. S.
8	6	1.05 ± 0.01	0.83	N. S.
9	16	1.10 ± 0.03	1.21	N. S.
Lung Tissue Culture				
Control	30	1.04 ± 0.01	----	----
Experimental				
1	5	1.09 ± 0.01	10.00	S. (0.05, 0.01)
2	5	1.10 ± 0.03	2.14	N. S.
3	5	1.06 ± 0.03	0.74	N. S.
4	6	1.08 ± 0.03	1.29	N. S.
5	5	1.05 ± 0.02	0.56	N. S.
6	5	1.05 ± 0.01	1.25	N. S.
7	5	1.10 ± 0.03	1.71	N. S.

[a] N.S. means that there is not a significant difference between experimentals and controls. S. (0.05) means that there is a significant difference at probability = 0.05. S. (0.05, 0.01) means there is a significant difference at probability = 0.05 and probability = 0.01.

CHAPTER IV

DISCUSSION

The induction of dominant lethality in the offspring of presterile-period matings of irradiated males has been documented in mice, rats, guinea pigs, rabbits, and golden hamsters among others (Regaud and Dubreuil, 1908; Strandskov, 1932; Snell, 1933; Hertwig, 1935; Hertwig and Brenneke, 1937; Henson, 1942; Amoroso and Parkes, 1947; Chang, Hunt, and Romanoff, 1957; Russell, 1954; and Lyon and Smith, 1971). Regaud and Dubreuil (1908) found degenerated and non-segmented ova in the fallopian tubes and sites of resorption in the uteri of rabbits mated with X-rayed males. Amoroso and Parkes (1947) and Chang, Hunt, and Romanoff (1957) observed that after in vitro irradiation of spermatozoa of rabbits most embryonic death occurred before implantation. Hertwig and Brenneke (1937) found evidence that abnormal, fertilized ova did not survive to implantation in the mouse; Snell (1933) attributed the lowering of the litter size in mice as due to resorption of abnormal embryos, since he found sites of resorption in the uteri. Russell (1954) observed that the effect of raising the dose to mice was to increase the ratio of preimplantation to postimplantation death. Lyon and Smith (1971) observed a genera difference in this ratio for a given dose; in rabbits, most of the prenatal death occurred before implantation, whereas in mice, guinea pigs, and golden hamsters, most of the prenatal death occurred after implantation. In the present work, evidence was found for resorption of

embryos in the Chinese hamster after paternal X-irradiation. Table 1 shows that 52 percent of the embryos were resorbed after an exposure of 400 r to their fathers.

Spontaneous translocations, as well as rings and dicentrics, occur very rarely. C. E. Ford (1964) reported that examination of 3313 mice, 181 Chinese hamsters, 220 common shrews, 31 rats, and 43 others revealed only one translocation in a shrew. In the study reported here there were no aberrations in the 12 Chinese hamsters used as controls. The observation of translocations in two of sixteen experimental animals studied would indicate that they were the result of radiation damage to the male gamete. The presence of translocations supports the extensive work done in mice by Snell (1933, 1935, 1946) and Russell (1954) using semi-sterility as an indicator of induced translocations. Translocations have also been observed in the presterile-period offspring of irradiated male golden hamsters (Lyon and Smith, 1971). Furthermore, translocations have been observed cytologically in the offspring of mice, rats, guinea pigs, and rabbits irradiated and mated so as to sample spermatozoa which were in the spermatogonial stage at irradiation (Griffen, 1958; Leonard and Deknudt, 1968; Lyon and Smith, 1971).

One chromosome was missing in the karyotype of one of the animals which had a translocation. The missing chromosome could have been one of the sex chromosomes or one of the members of pair 4; it is more likely that it was one of the sex chromosomes. It has been demonstrated that the X_2 and Y chromosomes of the Chinese hamster, mice, and man can be deleted *in toto* without accompanying lethality (Yerganian, 1963; Yerganian *et al.*, 1964). In mice the XO is a fully fertile female (Russell, 1961), whereas

cases reported in humans (Turner's Syndrome) are female with gonadal dysgenesis (Ford, et al., 1959). Russell has shown that XO mice can be produced by paternal X-irradiation (Russell and Saylors, 1962; Russell and Saylors, 1963).

If plant root tips are examined at the first metaphase after irradiation, various types and frequencies of chromosomal structural change are seen (depending on dose, intensity, type of irradiation, and the stage of the cell at the time of irradiation). Loss of fragments at subsequent mitoses renders many of these cells deficient; these deficient cells disappear from the mitotic population, if growth continues, leaving only apparently normal cells or clones of cells with balanced rearrangements (Sax, 1941; Ford, 1964; Wolff, 1968). Thus there is competitive as well as conservative selection at the cellular level.

Such mechanisms may operate in mammals also. Bender and Gooch (1961) irradiated Chinese hamsters and examined their bone marrow for aberrations; whereas all the expected types were found, the aberration yield fell rapidly with time. Further evidence for selection in mammalian organisms has been given by the radiation chimera work of C. E. Ford (1964). In his work mice are exposed to a lethal whole-body dose of X-rays and then injected intravenously with bone marrow cells from another mouse. The injected cells proliferate and replace damaged tissue. If cells from the bone marrow, spleen, thymus, and lymph nodes are sampled a few days after irradiation, there are numerous mitotic cells with aberrant karyotypes; thereafter they disappear rapidly. Eventually the new tissue may be replaced by growth of a few cells surviving from the original tissue. The majority of these cells have chromosome complements

indistinguishable from normal; the remainder have one or more abnormal chromosomes (some with longer or shorter arms, or with abnormal centromere positions). Commonly one or more cells are found with the same set of unique marker chromosomes (aberrant), indicating that these cells are derived by mitotic descent from a single radiation-damaged cell. In the zygote there should not be any competitive selection pressure although the fact that many aberration types (e.g., rings, dicentrics) are not recovered in the offspring of irradiated males would indicate either that conservative selection pressure is very great or that rings and dicentrics break at anaphase and that the broken ends heal, leaving chromosomes that may be longer or shorter (Sax, 1941; Russell, 1954; Lea, 1962; Ford, 1964; Leonard and Deknudt, 1968).

In certain exceptional cases, dicentrics and rings have been observed to persist past numerous mitotic divisions. In maize endosperm dicentrics are found many divisions past their inception, but this has been shown to be due to a breakage-fusion-breakage cycle (McClintock, 1939); i.e., the dicentric is broken at each cell division and the sister ends unite to form new dicentrics. Ring chromosomes are found to persist throughout the entire somatic cycle in maize although the small rings may remain in the cytoplasm at mitosis and be lost (McClintock, 1938). Rings and dicentrics (as well as deletions, pericentric inversions, and reciprocal translocations) have been found to persist in the peripheral leukocytes of irradiated humans; the leukocytes must have gone through mitosis since no fragments were found in the cells (Bender and Gooch, 1963). Rings associated with abnormal human phenotypes have been found to have high levels of persistence; a ring which resulted in the deletion

of ten percent of chromosome 1 was found consistently in 80 percent of the leukocytes examined over a three month period in a dwarf, microcephalic, mentally retarded child (Cooke and Gordon, 1965). Fracarro and Lindsten (1964) found in one case of Turner's Syndrome a self-perpetuating ring chromosome which was persistently present in periodic examinations of the subject's blood cultures; this ring was found with a lower frequency or not at all in other tissues examined. The majority of cells which did not have a ring chromosome were XO cells. In these cases either the frequency of bridge formation is low or the aberrant cells somehow enjoy a selective advantage; in maize endosperm the triploid condition may contribute to the decreased deleterious condition (Sax, 1941).

In the formation of a dicentric or ring, one would expect the loss of acentric fragments so that healing of the broken ends should result in chromosomes with shortened arms (Sax, 1941). If a breakage-fusion-breakage cycle took place, the breaks would not always occur at the same point so that daughter chromosomes would be produced with deficiencies and duplications (McClintock, 1938, 1939). The significant differences found by our measurements and statistical analysis could be the result of such processes. The loss of an entire chromosome could be the result of the loss of a dicentric or ring chromosome.

The data in Tables 2-5 are summarized in Table 6. The centromere index is shown to be a more sensitive indicator of chromosome damage than the homologue ratio. Although a larger sample would be needed to verify the frequency, the trend is obvious; it would be expected that the centromere index would be more sensitive since pericentric inversions and reciprocal translocations between arms of the same chromosome would affect

the centromere index but not the total length of the chromosome.

Table 6. Frequencies of Significant Differences for the Statistical Tests

	Chromosome Pair			
	1		2	
	Embryo	Lung	Embryo	Lung
Centromere Index				
Experimental--Frequency of Significant Differences*	0.22	0.29	0.44	0.43
Average Frequency for Each Chromosome Pair	0.26		0.43	
Sum of Average Frequency for Both Chromosome Pairs	0.69			
Homologue Ratio				
Experimental--Frequency of Significant Differences**	0.11	0.29	0.11	0.14
Average Frequency for Each Chromosome Pair	0.20		0.13	
Sum of Average Frequency for Both Chromosome Pairs	0.33			

*F-test

**T-test

It would be of interest to compare the frequency of abnormalities obtained by our statistical analysis with aberration frequencies found in the literature. To find the frequency of abnormalities per cell, one would have to divide the observed frequency by the fraction of the length of the total genome represented by the lengths of chromosomes 1 and 2 since

measurements were made only for chromosomes 1 and 2. Bender and Gooch (1961) found the fractional length of the total genome represented by chromosome pairs 1 and 2 to be 0.38; therefore,

$$\frac{\text{The frequency of abnormalities in chromosomes 1 and 2}}{\text{The fraction of total genome in chromosomes 1 and 2}} = \frac{0.69}{0.38} = 1.8$$

This would be the case if the incidence of breaks observed in different chromosomes is in a linear relationship to the chromosome length. The data of Wakonig and Ford (1960) show that more breaks occur in the long chromosomes than in the short (by more than a factor of two). Similarly they found that 65 percent of the chromatid intrachanges and exchanges occurred in the two longest chromosomes (Wakonig and Ford, 1960). Thus the value of 1.8 is probably overestimated by a factor of at least two. To compare the number of aberrations per cell found for diploid cells, our frequency would be multiplied by two since we irradiated haploid cells. Therefore the factors of two cancel, and we have a frequency of 1.8 abnormalities per cell for 400 r. A comparison with values found in the literature is given in Table 7.

Our analysis should pick up some abnormalities that are not scored in the references cited. In these references, aberrations are scored visually while looking at the cells under a microscope; no attempt is made to pair the chromosomes and examine them for small structural changes (changes in length, changes in centromere position); inversions also would not be scored. As discussed in the preceding paragraphs, cells with aberrant karyotypes may not survive because of competitive or conservative selection pressures. In the zygote, abnormal karyotypes may lead to

dominant lethality, and thus some of the aberration types scored in the references may be eliminated. Our study indicates, however, that extensive structural damage persists in the zygote and offspring.

Table 7. Radiation-induced Aberration Frequencies for the Chinese Hamster

Reference	Cell Type	Sampling Time Post-Irradiation	Aberrations per cell[a]	Aberrations Per Cell for 400 r
(1)	Primary Chinese hamster cell line derived from embryo	20 hours	0.0086 + 0.00194 R chromosome exchanges per cell[b]	0.7846 chromosome exchanges per cell
(2)	Bone marrow in vivo	6 hours	0.0021 r breaks per cell[c]	0.84 breaks per cell
(3)	Bone marrow in vivo	6 hours	0.015 + 0.0015 r breaks per cell[c]	0.615 breaks per cell
	Spermatogonia in vivo	6 hours	0.032 + 0.0041 r breaks per cell[c]	1.672 breaks per cell
(4)	Postmeiotic gametes (multiplied by two for diploidy)	15 days		1.8 abnormalities per cell

(1) Fetner (1970)
(2) Bender and Gooch (1961)
(3) Brooks and Lengemann (1967)
(4) Present study

[a] r = dose in roentgens; R = dose in Rads

[b] The number of exchanges per cell equals the number of rings plus the number of dicentrics per cell; this number would be one-half the number of breaks.

[c] The number of breaks per cell equals the number of chromatid deletions plus the number of isochromatid deletions plus twice the number of chromatid exchanges.

The fact that extensive structural damage was transmitted and a high frequency of dominant lethality was found indicates that the post-meiotic gametes of the Chinese hamster are very sensitive to X-ray-induced damage. In various strains of mice for a dose of 400 rads, Frölen found the frequency of resorption to vary from 29-31 percent the $LD_{50(30)}$ for these mice varied from 445 to 748 rads (Frölen, 1963). Russell (1954) found for mice that there was a 40 percent reduction in litter size (partially due to preimplantation death) for an exposure of 600 r. The golden hamster, $LD_{50(30)}$ 625 rads (Corbascio, Kohn, and Yerganian, 1962), shows an extreme sensitivity of mature sperm for a dose of 200 rads (Lyon and Smith, 1971); 30 percent dominant lethality was found for this dose (including preimplantation death). Doses of 500 rads yielded 27 percent dominant lethality in the guinea pig, 40 percent in the rabbit, and 40 percent in the mouse (Lyon and Smith, 1971). Thus, postmeiotic stages in the golden hamster and Chinese hamster are more sensitive to radiation-induced damage than several other mammalian genera.

CHAPTER V

CONCLUSIONS

A study of the chromosomes of cultured embryos and lung cultures of month-old offspring sired by male Chinese hamsters (Cricetulus griseus Milne-Edw.) given an acute, whole-body exposure of 400 roentgens with 250 KV X-rays has led to the following conclusions:

1. Sites of resorption were found in the uteri of females mated with X-irradiated males, but they were not found in the controls. It is probable that they were sites of resorption of defective embryos sired by X-irradiated males.

2. Reciprocal translocations were found in both an embryo culture and a lung culture of an offspring of X-irradiated male Chinese hamsters. These translocations resulted from X-ray-induced breaks in the fathers' gametes.

3. The loss of an entire chromosome was also observed in the lung culture from a month-old hamster sired by an X-irradiated male.

4. Statistical analysis of the centromere index and the homologue ratio of the first and second chromosome pairs revealed that there were significant differences between experimentals and controls in the morphology of these chromosomes. These changes were caused by X-ray-induced breaks in the fathers' gametes.

5. The centromere index is more sensitive than the homologue ratio as an indicator of radiation damage.

CHAPTER VI

RECOMMENDATIONS

Based on insight gained during the course of this investigation, the following recommendations are noted as being of future interest:

1. Offspring of irradiated males could be tested for the presence of induced translocations (without sacrificing the animal) in the following ways: (a) a male could be unilaterally orchidectomized, and spermatocytes examined for meiotic stages. Late pachytene could be examined for a cross-like configuration and mid-diplotene and diakenesis could be examined for a ring-of-four configuration indicating a reciprocal translocation; (b) a blood sample could be grown in cell culture, colchicine added, and mitotic metaphases observed. The cell type sampled would be the leukocyte.

Such procedures would insure the propagation of the translocation (assuming fertility of the animal). Thus, animals, which had been evaluated cytologically, could be tested genetically and for viability, sterility, and semi-sterility. Reciprocal translocation stocks would be very valuable for genetic analysis when genetic markers and linkage groups are determined for the Chinese hamster.

2. Experiments could be set up to determine the cause and frequency of embryonic death in embryos sired by irradiated males. Males would be irradiated, perhaps with an even higher dose than used in this study, since the $LD_{50(40)}$ is 856 r (Corbascio, Kohn, and Yerganian, 1962). Several factors could be varied to gain the most information as follows:

(a) the time between irradiation and mating could be varied to detect differences in stage sensitivity of the different spermatogenic stages; (b) the time of conception should be observed closely (i.e., the females should be observed to determine the time of estrus), and the females sacrificed at various intervals so that various stages of embryogenesis could be examined for aberrations. Females should be examined for corpora lutea, normal and degenerating ova, or live and dead fetuses so that an estimation of the death before and after implantation could be made. Squash preparations could be made of the early stages in an effort to determine aberrations that might be eliminated in future mitotic divisions.

APPENDICES

APPENDIX I

PREPARATION OF CELL CULTURE MEDIA

The culture media used for these studies is a modified form of that proposed by Eagle (1955, 1959) for the *in vitro* culture of mammalian cells. The composition of the media is given in Table 8.

Table 8. Components of Cell Culture Media

Compound	mgm/liter
Hank's Balanced Salt Solution	
NaCl	8000
KCl	400
$CaCl_2 \cdot 2H_2O$	186
$MgSO_4 \cdot 7H_2O$	100
$MgCl_2 \cdot 6H_2O$	100
$Na_2HPO_4 \cdot 7H_2O$	90
KH_2PO_4	60
Dextrose	1000
Phenol red	5
$NaHCO_3$	350
Minimum Essential L-Amino Acids	
Arginine	105
Cystine	24
Histidine	31

Table 8. (Continued)

Isoleucine	52
Leucine	52
Lysine	58
Methionine	15
Phenylalanine	32
Threonine	48
Tryptophan	10
Tyrosine	36
Valine	46
Glutamine	292
Growth-Promoting Amino Acids	
Asparagine	35
Glycine	15
Serine	20
Minimum Essential Vitamins	
Choline	1
Folic Acid	1
Inositol	2
Nicotinamide	1
Sodium pantothenate	1
Pyridoxal	1
Riboflavin	0.1
Thiamine	1
Others	
Sodium pyruvate	110
Penicillin "G" sodium	50
Dihydrostreptomycin sulfate	50
Fetal Calf Serum (Armour Co.)	200 ml/liter
2N NaOH	To adjust to pH 7.7
H_2O	To make to volume (1000 ml)

Water used to prepare the media had been twice distilled, and

then deionized on an Amberlite ion exchange column (Rohm and Haas resins IRA 400 and IR 120). A conductivity bridge (YSI-Model 31) was used to measure the conductivity of the water; water which gave a resistance reading of one million ohms or greater was acceptable for use in the media.

Glassware used in preparation of the media was boiled in a solution of Microsolv, scrubbed and rinsed seven times with distilled water and dried in an oven. The Millipore filter apparatus and bottles for cell culture were autoclaved and stored in a hood and oven, respectively, each of which had a germicidal lamp.

Stock solutions of the vitamins and amino acids (obtained from Nutritional Biochemicals, Cleveland, Ohio) were prepared in advance. The vitamins were dissolved in water and stored in sealed vials in a liquid nitrogen refrigerator. The amino acids, except for glutamine, were dissolved in 0.2 N HCl and kept frozen in a dry ice freezer. The first five compounds in Hank's Balanced Salt Solution were made up as one stock solution, and the next two as the phosphate buffer. All other compounds were added to the media separately. All stock solutions were prepared to yield the concentrations given in Table 8 when combined. The serum was filtered on a Seitz filter before it was added to the media. Sodium hydroxide (2 N) was used to adjust the pH to approximately 7.7 after the first five salts, amino acids, and phenol red indicator had been combined. Upon final mixing, the media was filtered through a Millipore filter (GS type; mean pore size, 0.22 microns) and stored in an incubator at 35°C under an atmosphere containing 5 percent carbon dioxide.

APPENDIX II

STATISTICAL ANALYSIS

The standard error of the mean ($s_{\bar{x}}$) was calculated as follows (Snedecor and Cochran, 1967):

N = sample size

$\bar{x}$ = mean

$x = (X - \bar{x})$ deviation from the mean

X = individual values

$$s^2 = \frac{\Sigma x^2}{(N-1)}$$

$$s_{\bar{x}} = \frac{s}{\sqrt{N}} = \frac{\sqrt{\frac{\Sigma x^2}{(N-1)}}}{\sqrt{N}}$$

$$s_{\bar{x}} = \frac{\sqrt{\frac{\Sigma X^2 - \frac{(\Sigma X)^2}{N}}{(N-1)}}}{\sqrt{N}}$$

(A) The value of t for the Student's t-test was calculated for the length ratio as follows (Snedecor and Cochran, 1967):

$\bar{x}$ = value to be tested

u = population mean

$$t = \frac{\bar{x} - u}{s/\sqrt{N}} \quad \text{with degrees of freedom } (N-1)$$

Values of t were compared with values given in a t-table for degrees of freedom equal to (N-1). Values of $\bar{x}$ and $s/\sqrt{N}$ which yielded t-values greater than or equal to those in the tables at P = 0.05, 0.01 were said to be significantly or highly significantly different from u. A two tailed t-test was used because the length ratio could be either increased or decreased by the treatment.

(B) An F-test was done on the centromere index (Snedecor and Cochran, 1967):

$$F = s_1^2 / s_2^2$$

where s_1^2 is the larger mean square. A one-tailed F-test was used with $df_1 = N_1\text{-}1$ and $df_2 = N_2\text{-}1$.

(C) An analysis of variance (Snedecor and Cochran, 1967) was run on the controls to make certain that there was not more variation within the animals than among them. The analysis of variance was performed as follows:

$$\text{between animals sum of squares} = \sum \frac{Tm^2}{Nm} - \frac{T^2}{N}$$

$$\text{error sum of squares} = \sum x^2 - \sum \frac{Tm^2}{Nm}$$

$$\text{total sum of squares} = \sum x^2 - \frac{T^2}{N}$$

where $T = \Sigma x$

$Tm = \Sigma x_m$

N = total number of items

m = sample

Table 9. Control Embryo-Centromere Index--Pair 1

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.0041447	5	0.0008289
Within animals	0.0304633	54	0.0005641
Total			0.000586
			F = 1.47 (N. S.)

Table 10. Control Embryo-Centromere Index--Pair 2

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.0039195	5	0.0007839
Within animal	0.0186415	54	0.0003452
Total			0.0003823
			F = 2.27 (N. S.)

Table 11. Control Lung-Centromere Index--Pair 1

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.0092612	5	0.0018522
Within animal	0.0437038	54	0.0008093
Total			0.000898
			F = 2.30 (N. S.)

Table 12. Control Lung-Centromere Index--Pair 2

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.0031891	5	0.0006378
Within animal	0.0174779	54	0.0003237
Total			0.000350
			F = 1.96 (N. S.)

Table 13. Control Embryo-Ratio--Pair 1

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.00765	5	0.00153
Within animal	0.04890	54	0.00204
Total			0.001958
			F = 0.74 (N. S.)

Table 14. Control Embryo-Ratio--Pair 2

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.0188	5	0.00376
Within animal	0.0596	54	0.00248
Total			0.0027
			F = 1.51 (N. S.)

Table 15. Control Lung-Ratio--Pair 1

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.0268	5	0.00536
Within animal	0.0605	54	0.00252
Total			0.00301
			F = 2.14 (N. S.)

Table 16. Control Lung-Ratio--Pair 2

Source	Sum of Squares	DF	Variance Estimate
Between animals	0.0060	5	0.0012
Within animal	0.04 5	54	0.0017
Total			0.00164
			F = 0.71 (N. S.)

(D) A t-test was also performed to determine whether there was a significant difference between the centromere index of control chromosome pair 1 and control chromosome pair 2. In both the embryo and lung control there was a highly significant difference.

$$t = \frac{\bar{x} - \bar{x}'}{s(\bar{x} - \bar{x}')}$$

$$s(\bar{x} - \bar{x}') = \sqrt{s^2 \left(\frac{1}{N} + \frac{1}{N'}\right)}$$

$$= \sqrt{\left[\frac{ss + ss'}{(N-1) + (N'-1)}\right]\left[\frac{1}{N} + \frac{1}{N'}\right]}$$

$$df = (N-1) + (N'-1)$$

Embryo: $t = \frac{0.471 - 0.445}{0.00403} = 6.45$ S. (0.05, 0.01)

Lung: $t = \frac{0.468 - 0.447}{0.00458} = 4.59$ S. (0.05, 0.01)

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