Table 1. FREQUENCY OF CHROMOSOME ABERRATIONS

	In vitro irradiation			*In vivo* irradiation						
Days after irradiation				78	170	189	275	276	1,673	2,698
Metaphase in cell cycle	1st	2nd	3rd							
Tricentrics	10	1	0	4	2	1	4	3	0	0
Dicentrics	240	100	23	114	65	42	82	93	18	2
Rings: centric	26	18	6	13	5	9	10	6	3	1
Rings: acentric, unpaired	22	2	0	12	5	11	11	11	2	0
Rings: acentric, paired	0	4	0	0	0	0	0	0	0	0
Acentric fragments: unpaired	440	3	0	190	100	85	115	130	24	2
Acentric fragments: paired	0	93	10	3	0	0	4	0	0	0
Abnormal monocentrics	88	50	23	46	25	26	34	31	13	7
Unstable cells with unpaired acentrics	179	0	0	88	64	62	67	65	10	2
Unstable cells with paired acentrics	0	71	10	2	0	0	1	0	0	0
Unstable cells without acentrics	3	43	19	2	2	0	2	1	0	1
Unstable cells with simple aneuploidy	2	13	11	4	1	4	2	1	2	3
Unstable cells with quasidiploidy	10	15	13	15	14	11	15	8	11	12
Unstable cells with normal karyotype	6	15	16	89	79	123	113	75	130	182
Number of cells observed	200	157	69	200	160	200	200	150	153	200

standard medium[3]. The cultures were treated with tritiated thymidine (specific activity 6·5 c./mmole, final concentration 1 μc./ml.) for a 20 h period commencing 25 h after the start of culture. (Investigations of the proliferation of lymphocytes from this donor showed that only a negligible number of cells entered the second *S* phase in culture before the end of the treatment period.) After this treatment the cells were washed in a medium containing cold thymidine and were then incubated for additional times in the standard culture medium supplemented with colcemide for the last 1·5 h of incubation. The cultures were terminated after either 72 h or 85 h. Chromosome preparations were made and subjected to autoradiography. After a 1 week exposure, the autoradiographs were developed and stained in Harris haematoxylin. Metaphase cells in which one of the two chromatids in each chromosome (second division) and approximately a quarter of the chromatids (third division) were labelled and their position on the slides was noted. Then cover slips were taken off in xylol and the slides were rehydrated by passing through an alcohol series. After desilverization in potassium ferricyanide fixer mixture, the emulsion was removed by soaking the slides in 70 per cent acetic acid for 3–24 h. The slides were then washed in tap water, stained in Giemsa and mounted in 'Permount'. Chromosome analysis was carried out on the previously selected metaphase cells. In addition, one of the cultures was treated with colcemide from 24 h and collected after 50 h; in this preparation all the metaphase cells are in the first division in culture[3]. Similar 50 h cultures were also made of lymphocytes obtained from the peripheral blood of patients who had received radiation therapy for cervical carcinoma.

The probability that a given aberration will survive the first or second division *in vitro* can be estimated roughly from the data shown in Table 1. For example, the number of dicentrics in a cell decreases from 1·2 at the first metaphase to 0·6 at the second and 0·3 at the third metaphase. Clearly, the probability is close to 0·5 that a dicentric will survive either the first or second division *in vitro*, and the probability is about 0·25 that it will survive both cell divisions. Similarly, the probability is about 0·9 that an abnormal monocentric chromosome will survive either of the first two cell divisions. The behaviour of the acentric fragments requires special comment. About 70 per cent are lost at the first cell division; the surviving 30 per cent, because of the lack of a centromere, are not distributed to the daughter cells, and they appear, therefore, as paired structures at the second metaphase and (sometimes) in groups of four at the third metaphase after irradiation.

The cells with chromosome aberrations can be grouped as unstable cells which contain polycentric chromosomes, rings, acentric fragments or are aneuploid; and stable cells which are either quasidiploid or have a normal karyotype. At the first metaphase the unstable cells are 92 per cent of the total, and all but 2 per cent of the cells contain unpaired acentric fragments. At the second and third metaphase the relative numbers of unstable cells drop sharply, and the acentric fragments are all paired. The probability that an unstable cell will survive division relative to the probability that a stable cell will survive division is about 0·35.

The results of the chromosome analysis in cells irradiated *in vivo* are shown in the table. About 48 per cent of the cells are unstable 2 months after irradiation. This frequency is about twice that reported earlier on the basis of data obtained with 72 h cultures[2]. (In such cultures we now know a large fraction of cells are in the second division[3].) The rate of decrease of unstable cells is not significantly different from that reported previously[2], and we estimate again a lifetime for lymphocytes of about 530 days. But even at 2,698 days after irradiation there are no unstable cells with paired acentric fragments; that is, the unstable cells decreased in frequency but gave no evidence of having divided since receiving radiation. A possible explanation for the failure to find such evidence of cell division is that the lymphocyte, when it is stimulated to divide *in vivo*, goes through several divisions. Based on the selection against unstable cells of 0·35, we calculate that at least four to five divisions must occur during lymphocyte production to account for the observed data. In support of this interpretation is the fact that the lymphocyte, after only a brief exposure to phytohaemagglutinin *in vitro*, will divide several times. There is supporting evidence in that production of rat thymocyte involves six to eight divisions[4,5]. Such a number of divisions will effectively eliminate the Cu cells. The selection pressure against quasidiploid cells, however, is much weaker, and the frequency of such cells changes very little during a period of many years.

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Attempts to induce Crossing-over in *Drosophila melanogaster* Males with Ovarian Extracts

Reddi, Reddy, and Rao[1] have reported results of an experiment in which crossing-over was induced in *Drosophila melanogaster* males by injection of a saline extract of the ovaries of *Drosophila*. This communication summarizes

the failure of two laboratories, operating independently, to repeat these experiments. In both laboratories efforts were made to adhere to the procedures described for preparation and injection of extracts. The assay systems were different and in both experiments they differed from that used by Reddi, Reddy and Rao[1].

The Vancouver experiment assayed crossing-over of chromosome 2 in three regions spread over a standard map distance of 56 units. Single treated males of the genotype *b cn c bw*/+ + + + were immediately crossed with three homozygous *b cn c bw* females. The males were transferred and given fresh females through three broods: 1–3 days, 4–6 days, and 7–10 days. Only broods 2 and 3 were scored.

The Storrs experiment examined recombination of chromosome 3 in five regions over a standard map distance of 70·7 units. Twenty-four hours after treatment single males of the genotype *ru h st* p^p *ss* e^s/+ + + + + + were individually mated to three homozygous *ru h st* p^p *ss* e^s females. After incubation for 4 days, the males were re-brooded every 3 days through a total of five broods. All broods were scored, but only the data for broods 2 and 3 are presented. No crossovers were found in other broods.

Table 1. ATTEMPTS TO INDUCE CROSSING-OVER IN MALE *Drosophila*

Laboratory	Treatment	Males treated	Treated males survival to brood 3	Total offspring in broods 2 and 3	No. of crossovers
British Columbia	Fresh extract	315	264	59,184	6
Connecticut	Fresh extract	127	92	9,790	2
	Boiled extract	111	73	7,416	0
	Saline	88	70	6,755	0
	Uninjected		112	12,415	7

Unfortunately, the results described in Table 1 do not confirm those of Reddi, Reddy and Rao[1], who report an overall induced crossover rate of 5 per cent. The rate observed in the present experiments (0·01 per cent) is less than that seen in the controls. It is surprising that Reddi, Reddy and Rao did not report any spontaneous cross-overs in rather extensive control samples.

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A New Marker for Chromosome Studies in the Mouse

THE mouse is one of the best mammals for genetic and cytogenetic investigations. Unfortunately, its value for cytogenetic research is restricted because all forty chromosomes are acrocentric. Their sizes range in the ratio of 5 : 2 (longest to shortest) with relatively little difference other than length to help in identification. At the first metaphase stage of meiosis, twenty bivalents are normally formed and this pairing is commonly used to correlate abnormal configurations with specific chromosome changes. It is obvious, however, that cellular markers (cells with a visible different karyotype from the normal) would be useful for transplantation experiments and work on carcinogenesis or radiation chimeras. Some translocations induced in mice by X-irradiation have been used as such cellular markers[1,2].

When comparing the radiosensitivity of different strains of mice[3], we were surprised to find that all the male germ cells at the diakinesis to first metaphase stages of control animals from the *AKR* strain maintained in our laboratory showed eighteen bivalents and one apparent quadrivalent (675 cells were analysed). The apparent quadrivalent was in the form of a ring in 672 cells and in the form of a chain in three cells. All the male offspring from translocated animals were found to have the translocation. Cytological investigations of bone marrow cells revealed that the number of chromosomes of male and female mice from the *AKR*/translocated strain is 38 : 36 acrocentric chromosomes and two metacentric chromosomes (Fig. 2). The total number of autosome arms is therefore the same as in the normal *AKR* strain[4] and the apparent quadrivalents can now be identified as metacentric bivalents. The metacentric chromosome is entirely autosomal because the sex chromosome bivalent is distinct at meiotic metaphase, as shown in Fig. 1. Measurements indicate that the arms of the metacentric chromosomes correspond approximately to the fourth and thirteenth autosomes of the normal complement taken in order of decreasing length. The stock of *AKR* strain in our laboratory is very small and all the animals showed the translocation. It was therefore impossible to be certain whether the translocation involved some alterations of the characteristics of the strain; however, as shown in Table 1, the

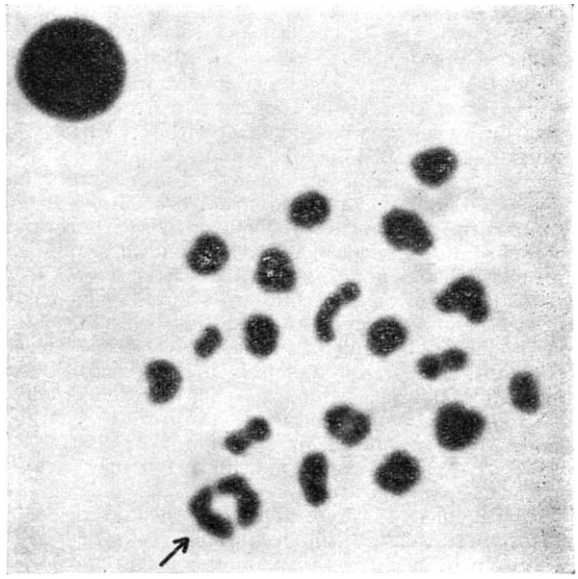

Fig. 1. Primary spermatocyte at metaphase (× 1,500).

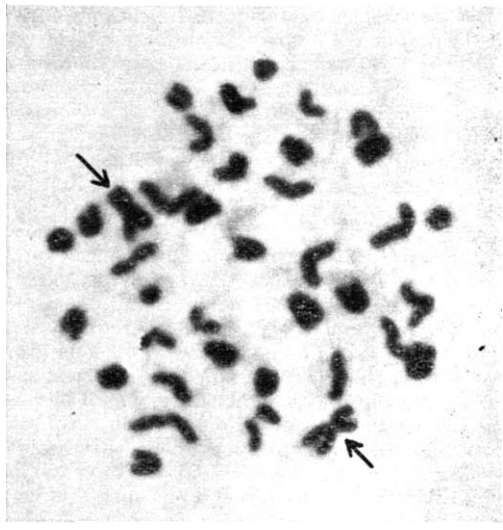

Fig. 2. Metaphase from bone marrow of male mouse (× 1,500).