SKELETAL ABNORMALITIES IN THE F_1 GENERATION OF MICE EXPOSED TO IONIZING RADIATIONS

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IN determinations of the genetic effects of radiation, the most nearly exact quantitative measurements can be obtained by using individual mutations (e.g. in the specific-locus mutation-rate studies) or certain classes of mutations. Such studies, however, although indispensable for comparative purposes, examine only parts of the overall genetic damage from radiation. At present, this damage must be measured empirically. The overall effect may be divided into categories which can be separately investigated by measuring parameters such as frequency of anomalies, survival after stress, learning capacity, percentage of mortality at various ages, fecundity, growth and development. Some are relatively easy to measure, but, since the vital characteristics may vary considerably from causes extraneous to the induced mutations, experiments must be controlled extremely carefully. Other characteristics, such as intelligence, are in themselves difficult to determine. Estimates of all parameters become even more complicated when one attempts to extend measurements beyond the F_1 generation of irradiated animals.

Important information has been obtained from empirical studies of morphological and physiological characters of descendants of exposed mammals. Decreased survival to weaning age in offspring derived from X-irradiated spermatogonia (RUSSELL and RUSSELL 1958), shortening of life in the offspring of male mice exposed to neutrons (RUSSELL 1957), reduction in the fertility of sons from irradiated males and females (SNELL 1935, 1946; HERTWIG 1938, 1941; RUSSELL 1950; RUSSELL and WICKHAM 1957), and indications of an increased frequency of morphological abnormalities derived from male mice exposed daily to X rays (CHARLES, TIHEN, OTIS and GROBMAN 1960) have been reported. This experiment was designed to test the feasibility of measuring part of the overall genetic damage by means of skeletal studies in the F_1 generation. A preliminary report of some of the results has been published (EHLING and RANDOLPH 1961).

MATERIALS AND METHODS

Male 101-strain mice were irradiated using different radiation sources and conditions. They were subsequently mated to C3H strain females and the

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progeny examined for skeletal abnormalities. Progeny were selected to sample gametes irradiated in different stages of spermatogenesis.

In the X-ray experiment the males were exposed to 600 rads (250 kvp, inherent filtration, 3 mm Al; HVL, 0.4 mm Cu; target-object distance, 84 cm). The intensity was 80 rads/min in one series and nine rads/min in another. For the exposure at 80 rads/min the males were confined in individual plastic tubes, and for the nine rads/min experiment in a partitioned Lucite wheel with a plastic cover. During irradiation the tubes and the wheel were rotating on a 10.8-cm-high masonite scatter block. The heads were shielded only in the nine rads/min experiment.

There were two fast-neutron irradiation conditions: (a) the testes of mice at about 15 cm from the target of a Cockcroft-Walton accelerator were irradiated at about five rads/hour by the 3.1 Mev neutrons produced in an almost forward direction by the D(d,n) He^3 reaction; (b) the testes of mice in three mm thick plastic tubes at about 13 cm from the target of the same accelerator were irradiated at about five rads/min by the 14 Mev neutrons produced at about 90° to the bombarding beam by the T(d,n) He^4 reaction. The dosimetry and latter target arrangement have been discussed earlier (CONGER, RANDOLPH, SHEPPARD and LUIPPOLD 1958; EDINGTON and RANDOLPH 1958).

Radiation conditions, number of males used, and number of first generation mice examined are summarized in Table 1. The males were 4–7 months old. Sham irradiations were used for the control mice. After irradiation, the males

TABLE 1

Experimental conditions

Mating period	Radiation			Dose (rad)	No. of males	No. of F_1 skeletons
	Type	Quality	Intensity (rad/min)			
Presterile	X ray	250 kvp	80	0	12	102
				600	16	49
		250 kvp	9	0	6	93
				600	12	72
	Neutron	14 Mev	5	0	6	124
				200	12	103
		3 Mev	0.08	0	6	63
				80	12	134
Poststerile	X ray	250 kvp	80	0	6*	236
				600	6*	157
All nonirradiated groups, total					30	618
All irradiated groups, total					52	515

* Males used earlier in presterile matings (corresponding group).

were mated to adult C3H females. Single-pair mating was used in the presterile period for the group of males exposed to X rays (80 rads/min) and for the contemporary control group. In all other groups, one male was mated to three adult females. For presterile period matings, the males were transferred to a fresh set of C3H females seven, 14, and 21 days after irradiation. Nonirradiated control males were mated in exactly the same manner. For poststerile matings, males remained with the same mates except that females were temporarily removed to give birth to and raise their litters.

All litters were recorded at birth and sacrificed when 26–28 days old. Animals were processed for skeletal study by a modification of Dawson's technique (DAWSON 1926) as follows: (a) skinning and evisceration, (b) hardening in 70 percent alcohol for 14 days, (c) maceration in several changes of one percent KOH, (d) staining in a slightly alkaline aqueous solution of Alizarin Red-S mixed with glycerine in a 4:1 ratio, and (e) clearing in pure glycerine. By this technique, all ossified parts of the skeleton are clearly stained red.

All skeletons were coded for examination in order to avoid any bias in the morphological judgment. The whole skeleton was examined in detail. References to cervical, thoracic, lumbar, sacral, and caudal vertebrae are occasionally abbreviated as CI, CII, . . . ; ThI, ThII, . . . ; LI, LII, . . . ; SI, SII, . . . ; and CaI, CaII, . . . ; respectively.

RESULTS

The duration of sterility of irradiated 101 males in our experiments is longer than the time reported by W. L. RUSSELL (1954) for (101 × C3H)F_1 males. This is mainly due to a later return to fertility following the sterile period (Table 2).

TABLE 2

Interval between irradiation and first poststerile conception

Irradiation*	Minimum time (days)	Median time (days)
600 X_H	98	104†
600 X_L	99	102
200 N_H	91	99
80 N_L	89	97

* X_H=X rays, 80 rad/min; X_L=X rays, 9 rad/min; N_H=neutrons, 5 rad/min; N_L=neutrons, 0.08 rad/min (see Table 1).
† Based on six males. All other groups based on 12 males.

The onset of sterility may also be earlier in the 101 males than in the hybrids, but this variable is not accurately measured by the mating system employed in this experiment. The differences are probably attributable to the strains of mice used. Strain differences in reproductive performance of females following irradiation are well-known (EHLING 1960).

Although litter size and sex at birth and at four weeks of age were recorded in this investigation, these data are not presented in detail since the experiment was not specifically set up for this purpose. The results could lead to wrong conclu-

sions if taken at face value and without further experimental analysis. Thus, survival between birth and four weeks of age is higher for the total of all irradiated groups (515 out of 543) than it is for all control groups (618 out of 691). However, in keeping with all earlier experiments, litter size at birth is greatly reduced in irradiated groups (as a result of dominant lethals), and the higher postnatal survival may well be the result of reduced competition.

Classification of morphological abnormalities

Morphological variations in the skeleton are greater in inbred strains than in F_1 hybrids between inbred strains (RUSSELL and RUSSELL 1954; GRÜNEBERG 1954). But even F_1 hybrids, as used here, though genetically uniform are not free of variability due to environmental causes. Hence, one of the main difficulties in the interpretation of the experimental results was to find some system of classification that would, at least partially, separate this existing natural variability from that caused by newly occurring genetic changes.

Since mutations are rare, the expected distribution of abnormalities in a very large experiment would be dichotomous with relatively rare and relatively common groups. Even so, identification of the mutational component would probably be imperfect because of overlap between parts of the distribution. Some of the rare abnormalities might be determined environmentally rather than mutationally, and some of the more common events might result from mutation at mutable loci or might be characteristics affected by mutation at many loci. Thus, the problem and goal are to divide the distribution such that an increase in mutations too small to affect the total frequency of abnormalities significantly may be detected by a statistically significant increase in the "relatively rare" class of abnormalities. In this experiment, where the sample size is such that not more than one mutation would be expected for any particular gene locus, it seemed the distinction could best be made by merely dividing the abnormalities according to whether they occurred only once in the whole experiment (Class 1) or more than once (Class 2). This classification was also used in the preliminary report (EHLING and RANDOLPH 1961). With this classification, a statistically significant increase in Class 1 abnormalities is expected to provide the most sensitive indication of mutation.

Animals having *Class-1 abnormalities* were subdivided as having multiple or single abnormalities. The multiple abnormalities were not all of Class-1 type; but, in order to be classified in this category, an animal had to have at least one Class-1 abnormality plus others, regardless of whether Class 1 or Class 2. The "Class 1-single" category was further subdivided according to whether the abnormalities were asymmetric (unilateral) or symmetric (either bilateral or central). All abnormalities of the vertebrae are tabulated as central. A more detailed description of Class-1 abnormalities is given in the following sections. Their frequencies in different series are summarized in Table 3.

Class-2 abnormalities were those found in two or more animals. Morphologically this class was subdivided into abnormalities of the vertebral column (Table 4), of the thorax (Table 5), and of the appendicular skeleton (Table 6).

TABLE 3

Frequency of Class-1 abnormalities

Stage	Irradiation*	No. of F_1 skeletons	Skeletons with multiple abnormalities	Skeltons with single abnormalities: Bilateral or central	Skeltons with single abnormalities: Unilateral	Total
	0	102	0	0	0	0
	600 X_H	49	1	3	1	5
	0	93	0	0	2	2
Postspermatogonial	600 X_L	72	1	4	1	6
	0	124	0	0	2	2
	200 N_H	103	0	1	4	5
	0	63	0	1	0	1
	80 N_L	134	1	1	2	4
	0	236	0	1	2	3
Spermatogonial	600 X_H	157	1	1	0	2
All control groups		618	0	2	6	8
		Percent	0	0.3	1.0	1.3
All irradiated groups		515	4	10	8	22
		Percent	0.8	1.9	1.6	4.3

* 0 = control group; for explanation of other symbols see footnote to Table 2.

TABLE 4

Frequency of Class-2 abnormalities of the vertebral column

Stage	Irradiation	No. of F_1 skeletons	Vertebral fusions	Dyssymphysis: CVII	ThI	ThX	ThXI	Processus spinosus, Additional dystop. absent: CIII	ThIII	ThIII	ThII	ThIX
	0	102	2	0	1	29	1	0	0	0	2	1
	600 X_H	49	0	0	1	11	1	0	0	0	0	2
	0	93	1	1	0	24	0	2	0	0	4	4
Postspermatogonial	600 X_L	72	0	0	0	28	1	0	1	0	0	2
	0	124	2	0	0	32	0	0	1	0	6	2
	200 N_H	103	1	0	0	17	0	0	0	2	3	2
	0	63	0	0	1	20	0	0	0	0	1	0
	80 N_L	134	0	0	0	28	0	0	0	0	6	0
	0	236	2	2	1	57	0	1	0	1	9	2
Spermatogonial	600 X_H	157	1	1	0	35	0	2	0	0	2	2
All control groups		618	7	3	3	162	1	3	1	1	22	9
		Percent	1.1	0.5	0.5	26.2	0.2	0.5	0.2	0.2	3.6	1.5
All irradiated groups		515	2	1	1	119	2	2	1	2	11	8
		Percent	0.4	0.2	0.2	23.2	0.4	0.4	0.2	0.4	2.1	1.6

TABLE 5

Frequency of Class-2 abnormalities of the thorax

Stage	Irradiation	No. of F_1 skeletons	Sternum			Costa	
			Malformation	Fusion	Bridge	Irregular articulation	Fracture
Postspermatogonial	0	102	0	2	0	0	12
	600 X_H	49	0	0	0	0	7
	0	93	0	1	0	0	11
	600 X_L	72	0	1	1	0	9
	0	124	0	0	0	0	17
	200 N_H	103	1	1	2	1	13
	0	63	1	1	0	0	4
	80 N_L	134	1	2	1	0	15
Spermatogonial	0	236	2*	4	1	1	19
	600 X_H	157	0	0	1	1	10
All control groups		618	3	8	1	1	63
		Percent	0.5	1.3	0.2	0.2	10.2
All irradiated groups		515	2	4	5	2	54
		Percent	0.4	0.8	1.0	0.4	10.5

* Descendants from the same male.

TABLE 6

Frequency of Class-2 abnormalities of the appendicular skeleton

Stage	Irradiation	No. of F_1 skeletons	Fore limb		Hind limb		
			Clavicle	Phalanges	Pelvis	Fibula	Phalanges
Postspermatogonial	0	102	2	0	0	0	2
	600 X_H	49	0	1	0	0	0
	0	93	0	0	0	1	2
	600 X_L	72	0	0	0	0	1
	0	124	0	0	0	0	1
	200 N_H	103	0	2	1	0	0
	0	63	1	0	0	0	0
	80 N_L	134	0	1	1	0	0
Spermatogonial	0	236	0	2	1	1	0
	600 X_H	157	0	1	0	0	0
All control groups		618	3	2	1	2	5
		Percent	0.5	0.3	0.2	0.3	0.8
All irradiated groups		515	0	5	2	0	1
		Percent	0	1.0	0.4	0	0.2

Description and frequency of abnormalities

Animals with Class-1 multiple abnormalities:[3] (a) 7-X_H, male, fusion of thoracic vertebrae V + VI, malformation of dystopia tubercoli anteroris (left), articulation of rib VIII with sternum (left), malformation of distal phalanges I (right forefoot), dyssymphysis of thoracic vertebra X; (b) 7-X_L, male, dyssymphysis of atlas, malformation of axis, 14 thoracic vertebrae, ThXII without surface structure, 14 ribs, articulation of rib VIII with the sternum, fusion of sternal plates IV + V, irregular articulation of ribs with sternum; (c) 7-N_L, female, dyssymphysis of atlas and thoracic vertebrae I + II, processus spinosus smaller than normal and shifted upon ThIII, 14 thoracic vertebrae, fusion of sacral vertebrae I + II, 14 ribs left, 13 ribs right, articulation of rib VIII with the sternum (left), six sternal plates; and (d) g-X_H, male, 14 thoracic, six lumbar, five sacral and 30 caudal vertebrae, dyssymphysis of ThVI, extremely close connection of ThX, ThXI, and ThXII, asymmetrical LVI, fusion of SI + II, 14 ribs, articulation of rib VIII with the sternum, fusion of the ventral segments of ribs I + II (left) articulation of ribs with sternum irregular.

Animals with Class-1 single abnormalities (bilateral or central): (a) 7-X_H, male, asymmetrical axis; (b) 7-X_H, male, malformation of processus spinosus (ThII); (c) 7-X_H, male, short tail (23 caudal vertebrae); (d) 7-X_L, male, malformation of rib I; (e) 7-X_L, female, incomplete ossification of thoracic vertebra VI; (f) 14-X_L, male, malformation of distal phalanges V (forefeet); (g) 14-X_L, male, fusion and malformation of caudal vertebrae XXVII + XXVIII; (h) 7-N_H, male, processus spinosus on CVII; (i) 7-C, female, dyssymphysis of ThII; (j) 21-N_L, male, malformation of xiphisternum; (k) g-C, female, foramina transversia imperfecta (CI); (l) g-X_H, defective development of CaXXVII.

Animals with Class-1 single abnormalities (unilateral): (a) 7-X_H, male, one additional carpal bone (left); (b) 14-C, female, abnormal epiphysis of calcaneus (left); (c) 21-C, male, proximal fibular epiphysis and corresponding part of fibula wider than normal (right); (d) 7-X_L, male, undersized 13th rib (left); (e) 21-C, male, swelling of distal part of the femur (right); (f) 21-C, female, apophysis of the scapular spine (left); (g) 7-N_H, male, 12 ribs right; (h) 7-N_H, female, abnormal proximal fibular epiphysis (right); (i) 21-N_H, male, middle lacerated foramen of basisphenoid is extremely small (left); (j) 21-N_H, male, enlarged ischium, pubis smaller than normal (right); (k) 7-N_L, female, malformation of hamate (right); (l) 7-N_L, female, bony outgrowth from mandible, inside (left); (m) g-C, female, fusion of radius and ulna (right); (n) g-C, male, apophysis of calcaneus (right).

Class-2 abnormalities of the vertebral column: The frequencies of these abnormalities in different series are summarized in Table 4. Fused vertebrae were found in thoracic, lumbar, and sacral regions. The following vertebrae were affected in the different groups: in controls, ThII + III, (three times); LV + VI;

[3] Symbols indicate mating period (7 = 1–7 days, 14 = 8–14 days, 21 = 15–21 days, g = spermatogonia) and experimental conditions (X = X-ray, N = Neutron, C = sham-irradiated control, H = high intensity, L = low intensity, see Table 1).

LVI + SI; SI + II; SII + III; in the N_H group, LVI + SI; in the g-X_H group, LIV + V. Four types of fusions were observed only once (LIV + V; LV + VI; SI + II; SII + III). However, since LV and SII were each involved twice, these four different types of fusions were counted as Class-2 abnormalities. No atlas-axis fusion or cervical fusions were found, although the frequency of this type of fusion was high in the strains used by H. GRÜNEBERG and co-workers for skeletal studies (GRÜNEBERG 1950; SEARLE 1954; DEOL, GRÜNEBERG, SEARLE and TRUSLOVE 1957). The frequency of dyssymphysis is low for cervical vertebra VII and thoracic vertebrae I, and XI, but is high for ThX. According to A. G. SEARLE (1954) the manifestation of dyssymphysis of the first thoracic vertebra is influenced by littersize, smaller litters having a higher proportion of affected mice. M. S. DOEL *et al.* (1957) also noted a very high frequency of ThX dyssymphysis and a significant difference between the sexes in the manifestation of this character. The incidence is higher in females. In the mouse, ThII commonly has a very conspicuous processus spinosus. The processus spinosus can vary from a very large structure to an absence. The distribution of sizes differs greatly from strain to strain (GRÜNEBERG 1950). In a few cases we found an additional processus spinosus either on the third cervical vertebra or on ThIII. In other cases, the prominent processus spinosus usually found on ThII had shifted caudally on to ThIII (dystop. ThIII in Table 4). As abnormalities of ThIX we noted an irregular shape of the posterior articular process (Table 4).

Class-2 abnormalities of the thorax: This group of abnormalities has been subdivided into malformation of the sternum, fusions, and bridges between sternal plates, irregular articulation of the ventral segments of the ribs, and fractured ribs (Table 5). Under the heading "malformation of the sternum" we tabulated mice with malformed sternal plates. The malformation of a sternal plate can be associated with irregularities of the whole sternum and of the costo-sternal articulation. The observed intrasternal fusions occurred only between two or three sternal plates. We never saw broad fusions described by L. B. RUSSELL (1956) after treatment in the embryonic stages (9½ and 10½ day). Fusions between sternebrae IV + V were observed ten times; between IV + V + VI, I + II, II + III once each. The term "bridge" was applied to a double stranded bony tissue connection between sternal plates. Observed bridges were between sternebrae III + IV + V, IV + V, and V + VI. Normally, thoracic ribs I–VII are "true" or vertebro-sternal ribs. In a few cases the eighth rib also was unilaterally fused with the sternum (Table 5, irregular articulation).

Class-2 abnormalities of the appendicular skeleton (Table 6): Unilateral manifestation is typical for abnormalities observed in this group with the sole exception of dyssymphysis of os ischium and os pubis. Abnormalities of clavicle, fibula, or phalanges consist of thickening of part of the bone. This thickening affects the distal region of the fibula and the acromial end of the clavicle. In three animals, os ischium and os pubis either failed to coalesce or were fused in an anomalous way. No other anomalies were observed in the pelvis.

Variable characters

Characters with great variability (the number of cervical ribs, the shape of the free end of the xiphisternum, the size of the third molar, the numbers and location of foramina hamate, the complete or partial division of the proximal fibular epiphysis) are excluded from our tabulation of Class-2 abnormalities. Furthermore, there is a high incidence of an extra element between sternebra V and the xiphisternum. This sternebra VI is visible from the ventral side of the sternum, but rarely can it be seen in dorsal aspect. The extra sternebra varies extremely in size and is always considerably smaller than the other sternebrae. It is usually attached to sternebra V.

The os innominatum in the mouse may articulate with the 26th, 27th, or 28th vertebra. Whichever of these vertebrae articulates with the pelvis assumes the shape and characteristics of the first sacral vertebra. Seven of the 25–27 presacral vertebrae that a mouse may have are invariable cervicals. There may be 12–14 thoracic vertebrae and 5–7 lumbar vertebrae (GRÜNEBERG 1952). The ratios of lumbar/sacral vertebrae in the different series of our experiment are given in Table 7. All changes, except in one case, were homeotic, i.e. border shifts, with the total number remaining constant (lumbar + sacral = 10). The one non-homeotic case, with a 6 6/5 5 formula was classified as Class-1 abnormality (see d under animals with Class-1 multiple abnormalities). For the homeotic cases, it may be noted that there is a distinct difference between left and right in the sacralization of the sixth lumbar vertebra.

TABLE 7

Distribution of lumbar to sacral vertebrae

Stage	Irradiation	No. of F_1 skeletons	6 6* / 5 5	6 6 / 4 4	6 5 / 4 5	5 6 / 5 4	5 5 / 5 5
Postspermatogonial	0	102	0	34	24	7	37
	600 X_H	49	0	17	15	2	15
	0	93	0	22	25	12	34
	600 X_L	72	0	29	15	3	25
	0	124	0	34	37	6	47
	200 N_H	103	0	32	31	2	38
	0	63	0	23	19	3	18
	80 N_L	134	0	54	28	2	50
Spermatogonial	0	236	0	70	79	11	76
	600 X_H	157	1	53	39	8	56
All control groups		618	0	183	184	39	212
		Percent	0	30	30	6	34
All irradiated groups		515	1	185	128	17	184
		Percent	0.2	36	25	3	36

* The numbers above and below the line represent the number of lumbar and sacral vertebrae, respectively. Left and right sides of the vertebral column are indicated by the corresponding sides of the formula.

In preparing the skeletons for study, some very small mice were observed. Since, however, animals were not systematically weighed, no accurate data on this point can be given. It is possible that the frequency of dwarfism is higher in descendants of irradiated males. For the rat, G. F. MACGREGOR and H. B. NEWCOMBE (1961) reported that the frequency of dwarfism is correlated with the radiation exposure accumulated over successive generations.

CONCLUSION

The results indicate that part of the overall genetic damage from irradiation can be detected by studies of skeletal abnormalities in the first generation. The distinct and statistically significant increase of Class-1 abnormalities (i.e. abnormalities observed only once in the whole experiment) in offspring from irradiated males suggests that the majority of these anomalies is due to induced dominant mutations. A pooling of all Class-1 abnormalities for all irradiated groups, *versus* all controls, yields a highly significant difference ($\chi^2 = 8.56$, $P = 0.004$). It may also be noted that all cases of Class-1 *multiple* abnormalities occurred in offspring of irradiated fathers ($P = 0.04$ for Class-1 multiples alone).

On separately examining the Class-1 abnormality data for offspring from spermatogonial and postspermatogonial irradiation, one notes that the effect is due to the latter only. The pooled frequency of Class-1 abnormalities from irradiation in the postspermatogonial stage is very significantly different from the incidence of abnormalities in the controls ($\chi^2 = 13.5$, $P = 0.002$). On the other hand, no significant difference in the incidence of Class-1 abnormalities could be observed to date between offspring from irradiated spermatogonia and controls. However, it is suggestive that one of the Class-1 multiple anomalies occurred in an offspring derived from an irradiated spermatogonium.

The postspermatogonial results, as far as they go, do not indicate any great effect of dose rate or LET. Thus, when frequency of Class-1 abnormalities is plotted merely according to dose (Figure 1), the various points, which represent different dose rates and LET's, fall roughly in a straight line, the slope of which differs very significantly from zero ($t = 8.58$, $P = < 0.025$). The 90 percent confidence intervals at each point are, however, wide. It should be noted that other types of genetic experiments, namely, specific-locus and dominant-lethal studies, have already shown that there is little or no dose-rate effect in postspermatogonial stages (RUSSELL, RUSSELL and KELLY 1958).

No differences in the frequency of Class-2 abnormalities between offspring derived from irradiated and nonirradiated males were observed. While it is possible that the number of skeletons examined was not large enough to detect such differences, it appears more likely that the majority of Class-2 abnormalities are the result of nongenetic factors.

Differences in the percentage of types of sacralization between descendants of irradiated and nonirradiated males ($P = 0.013$) are probably attributable to exogenous factors. W. L. RUSSELL (1948) demonstrated that sacralization is influenced by the maternal environment. Differences in the prenatal environ-

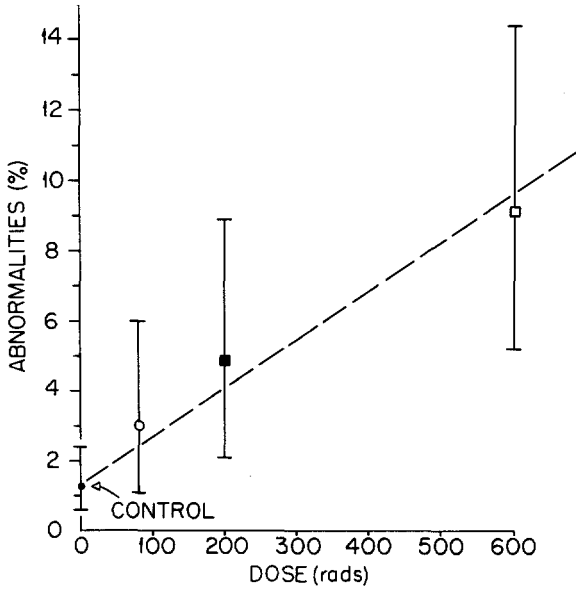

FIGURE 1.—Frequency of Class-1 abnormalities in offspring of males irradiated in post-spermatogonial stages, *versus* absorbed dose. Ninety percent confidence intervals. □ X rays, combined results; ■ 14 Mev neutrons; ○ 3 Mev neutrons; ● controls, combined results. The broken line is the weighted least squares regression fit to the data.

ment of offspring of irradiated and nonirradiated males would be expected to result from early embryonic death (due to dominant lethals) of many litter mates in the former group.

A comparison of abnormalities described by H. GRÜNEBERG and co-workers (GRÜNEBERG 1950, 1954; SEARLE 1954; DEOL *et al.* 1957) in papain-digested skeletons with our observations on the whole skeletons shows some differences in the types of abnormalities observed, probably due to the different preparation methods employed. Papain-digestion was not possible for our study since, with this method, the mouse should be at least six weeks old (GRÜNEBERG 1950). Differences in the frequencies of some kinds of abnormalities observed by GRÜNEBERG and by the present authors are partly attributable to different strains used.

SUMMARY

This experiment was designed to test the feasibility of measuring part of the overall genetic damage of irradiation by means of skeletal studies in the F_1 generation. Male 101-strain mice were irradiated, using different radiation sources and conditions, and subsequently the males were mated to adult C3H-strain females. Progeny were selected to sample gametes irradiated in different stages of spermatogenesis. The F_1 generation was sacrificed at 26–28 days of age and animals were processed for skeletal study by a modification of DAWSON's technique. Observed abnormalities were classified according to whether they

occurred only once in the whole experiment (Class 1) or more than once (Class 2). For Class-1 abnormalities, a highly significant increase in frequency was observed in offspring of irradiated males. This increase is due largely, if not wholly, to irradiation in postspermatogonial stages. For Class-2 abnormalities, no differences between irradiated and control groups could be detected. Therefore, we conclude that the classification used served its original purpose of distinguishing between the effects of newly occurring dominant genetic changes and variability from environmental causes. With the methods used, studies of skeletal abnormalities in the first generation can detect part of the overall genetic damage from irradiation.

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