References and Notes

1. M. Blau, E. S. Deevey, M. S. Gross, *Science* **118**, 1 (1953).
2. E. S. Deevey, M. S. Gross, G. E. Hutchinson, H. L. Kraybill, *Proc. Natl. Acad. Sci. U.S.* **40**, 285 (1954).
3. H. R. Crane, *Science* **124**, 664 (1956).
4. T. A. Rafter, *New Zealand J. Sci. Technol.* **37**, 20 (1955).
5. R. Revelle and H. E. Suess, *Tellus* **9**, 18 (1957).
6. H. R. Crane and J. B. Griffin, *Am. J. Sci., Radiocarbon Suppl.* **1**, 173 (1959).
7. W. S. Broecker and E. A. Olson, *ibid.*, p. 111.
8. ———, *Radiocarbon* **3**, 176 (1961).
9. W. S. Broecker and A. Walton, *Geochim. Cosmochim. Acta* **16**, 15 (1959).
10. H. Godwin, *Am. J. Sci.* **249**, 301 (1951).
11. M. M. Leighton, *J. Geol.* **68**, 529 (1960).
12. M. Rubin, R. C. Likins, E. G. Berry, *ibid.* **71**, 84 (1963).
13. P. Baertschi, *Nature* **168**, 288 (1951).
14. H. Craig, *J. Geol.* **62**, 115 (1954).
15. S. Oana and E. S. Deevey, *Am. J. Sci.* **258A**, 253 (1960).
16. M. L. Keith, R. Eichler, R. H. Parker (abstr.) *Geol. Soc. Am.*, Program 73rd Ann. Meeting, 133 (1960).
17. M. L. Keith and G. M. Anderson, *ibid.* 75th Ann. Meeting, 85A (1962).
18. C. R. McKinney, J. M. McCrea, S. Epstein, H. A. Allen, H. C. Urey, *Rev. Sci. Instr.* **21**, 724 (1950).
19. H. Craig, *Geochim. Cosmochim. Acta* **12**, 133 (1957).
20. H. Oeschger, U. Schwarz, C. Gfeller, *Am. J. Sci., Radiocarbon Suppl.* **1**, 133 (1959); E. A. Olson and W. S. Broecker, *ibid.*, p. 1.

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22. W. F. Libby, *Radiocarbon Dating* (Univ. of Chicago Press, Chicago, ed. 2, 1955).
23. H. Godwin, *Nature* **195**, 984 (1962).
24. W. F. Libby, *Science* **140**, 278 (1963).

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Radiocarbon Activity of Shells from Living Clams and Snails

As a logical extension of an artificial tracer study on snail shells (*1*), measurements were made of the natural carbon-14 activity of shells from living clams and snails collected from a variety of environments. Three samples were measured, representing growth in lime-poor, fairly soft, and lime-rich waters (Table 1). Samples were collected by D. W. Taylor in September and October 1959, and measured at the U.S. Geological Survey's radiocarbon laboratory. Sample W-1003 (*2*), from lime-poor water, had approximately 10 percent less C^{14} than the conventional modern standard (95 percent N.B.S. oxalic acid standard); W-1006 (*3*), from fairly soft water, was 22 percent deficient; and W-1004 (*4*), from lime-rich water, was 32-percent deficient. These deficiencies correspond to errors of approximately 800, 2000, and 3000 years if no correction is made for initial activity in computing sample ages. These measurements agree essentially with those of Broecker and Walton (*5*) and Keith and Anderson (*6*).

Table 1. Radiocarbon activity of shells.

Sample No.	Environment	δC^{14} (per mil)
W-1003	Spring pool in basalt, lime-poor water	−96.6
W-1006	Snake River, fairly soft water	−224.9
W-1004	Portneuf River, in limestone area, lime-rich water	−323.3

The tracer study (*1*) indicated that a snail can incorporate 10- to 12-percent inorganic carbon in making its shell and "the remainder of the carbon in the shell carbonate must come from food or the atmosphere, and the resulting C^{14}/C^{12} ratio will be essentially the same as in those sources." The control laboratory snails ate "modern" food (lettuce), whereas the natural snails ate food with a C^{14}/C^{12} ratio similar to their environment and so showed the greater deficiency in C^{14}. The limiting value of a system in contact with the atmosphere is 50-percent "dead" carbon, theoretically possible, but not yet measured anywhere. Even such a great deficiency in C^{14} as this will produce a constant error in the dating of unknown nonmarine carbonate samples of one C^{14} half life (5568 or 5730 years). Of greater significance is the postdepositional alteration and replacement of the original shell composition, which can add or subtract material. Neither visual nor mineralogic criteria can be used to predict the degree of this alteration (*7*). This can be determined only by mass spectrometric isotopic studies (*8*).

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References and Notes

1. M. Rubin, R. C. Likins, E. G. Berry, *J. Geol.* **71**, 84 (1963).
2. *Lithoglyphus hindsii* (Baird), from NW¼ sec. 17, T. 6 S., R. 13 E., Gooding County, Idaho.
3. *Gonidea angulata* (Lea), from NE¼ sec. 4, T. 6 S., R. 8 E., Elmore County, Idaho.
4. *Lithoglyphus hindsii* (Baird) SW¼ sec. 26, T. 7 S., R. 36 E., Bannock County, Idaho.
5. W. S. Broecker and A. Walton, *Geochim. Cosmochim. Acta* **16**, 15 (1959).
6. M. L. Keith and G. M. Anderson, *Science* **141**, 634 (1963).
7. K. K. Turekian and R. L. Armstrong, *Bull. Geol. Soc. Am.* **72**, 1817 (1961).
8. Publication authorized by the director, U.S. Geological Survey.

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Mutations: Incidence in Drosophila melanogaster Reared on Irradiated Medium

Abstract. *An increase in the rate of mutation has been found in* Drosophila melanogaster *reared on a basic medium that was irradiated with a sterilizing dose (150,000 rads) of cobalt-60 gamma rays. In Muller-5 tests, sex-linked recessive lethals occurred only in the F_2 progenies of the male test flies obtained from breeding the parent flies on irradiated medium, while visible changes occurred in experimental cultures from both the control and irradiated media. The frequency of sex-linked recessive lethals was 0.35, 0.55, and 0.8 percent in three independent experiments. Visible changes were two to six times more frequent in the irradiated series than in the controls.*

Earlier studies on the effects of irradiated culture media (White's basic medium, potato mash, and fruit juices) on mitosis in root meristems of *Hordeum,* sp. (barley), *Vicia faba* (broadbean), and *Allium cepa* (onion) have revealed that the products of such irradiation may have radiomimetic effects (*1*). In view of the obvious bearing of these data on assessing the wholesomeness of food sterilized by irradiation, we studied *Drosophila melanogaster* to ascertain whether there is an increase in mutations in flies that are fed irradiated food.

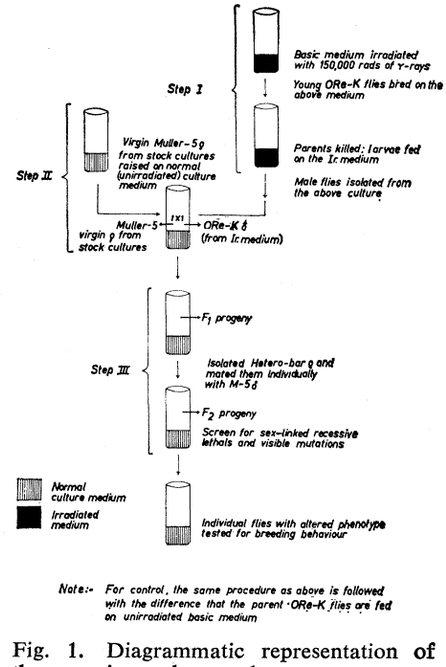

Fig. 1. Diagrammatic representation of the experimental procedure.

Table 1. Frequency of occurrence of sex-linked recessive lethal and visible phenotypic changes in F_2 families in Muller-5 tests.

Expt. No.	F_2 families					No. of flies		
	No. scored	Recessive lethals		Visible changes		Total scored	Mutants	
		No.	%	No.	%		No.	%
				Control medium				
1	352	0	0	6	1.7	34,945	6	0.018
2	542	0	0	13	4.08	16,923	16	0.095
3	480	0	0	32	6.60	11,416	36	0.315
				Irradiated medium				
1	830	3	0.35	65	7.8	81,432	102	0.13
2	544	3	0.55	72	18.0	25,324	93	0.37
3	640	5	0.8	78	12.2	17,716	87	0.497

Our procedure is represented in Fig. 1. The vials of basic medium (containing initially water, glucose, agar, yeast, and propionic acid in the proportion of 100:10:3:10:0.4 in grams) were arranged around a cobalt-60 source (160 c) and irradiated for 18 hours. The total dose was approximately 150,000 rads. In experiments on the extent of fungal contamination in irradiated and unirradiated culture media, treatment with 150,000 rads kept the medium free of contamination for a fortnight at 25°C and 50-percent relative humidity.

Young Oregon-K flies (Ore-K), taken soon after emergence, were transferred to the medium within an hour after it was irradiated. They were allowed to breed and the parent flies were killed after the eggs had been laid. The development of larvae on the irradiated medium was similar to that in the control medium, and the male flies emerging from the larvae that were reared on the irradiated medium were normal both in phenotype and growth. Male flies of similar age from the control and irradiated media were used in Muller-5 tests (Fig. 1). The experiment was repeated three times and the data are given in Tables 1 and 2. As can be seen from the data, sex-linked recessive lethals occurred only in progenies derived from the flies reared on the irradiated medium. The suspected lethal cultures were kept until no more flies hatched, and only those showing complete absence of Ore-K (+ type) flies were scored as lethals. All of the lethals were retested. Since the other three expected types of flies occurred in normal proportions, nondisjunctionally produced males do not seem to be involved. It thus seems reasonably certain that the recessive lethal factor arose in the X chromosomes of the Ore-K males reared on the irradiated medium.

A wide range of phenotypic changes was a striking feature of the F_2 flies from the irradiated medium series. Some of the mutants, such as curly wing and another dominant wing mutation, were retested and were found to breed true. Yellow body character was sex-linked when tested on mating with attached — X females. A few others, namely balloon, wingless, and bobbed, were completely sterile. Changes such as half-thorax, rotated abdomen, and absence of neck belong to the noninherited group of abnormalities (*2*). Nevertheless, it seems interesting that these mutations were relatively more preponderant in the irradiated medium series. Other noninherited abnormalities were mosaics, such as those with one wing normal and the other either curly, vestigial, or cut.

Table 2. Types and frequency of visible mutations observed in F_2 families (pooled data).

Mutant phenotype*	Mutations	
	Control medium	Irradiated medium
Heritable changes		
Curly wings	2	24
"Pad-like" wings (dominant)	0	3
Yellow body	1	18
Dumpy	0	2
Other changes†		
Balloon	4	15
Apterous	0	23
Bobbed	0	2
Jaunty	1	10
Ski	2	12
Vestigial wings	2	16
Absence of neck	2	1
Rotated abdomen	1	2
Half thorax	0	6
Strap	0	5
Club	7	8
Clipped	0	6
Incurved wings	3	13
Cut wings	0	5
Mosaics		
One wing	0	4
One wing curly and one normal	5	69
One wing vestigial and one normal	6	27
One wing vestigial and one curly	0	4
One wing cut and one normal	7	8

* The mutants were classified on the basis of their phenotypic appearance. Allelic tests are in progress wherever the mutants were found breeding true. † Includes: changes associated with sterility, changes not tested for heritability, and nonheritable changes.

Care was taken to insure that the only variable introduced was irradiation of the medium. Similar observations in three independent experiments indicate that irradiated media have a mutagenic effect. Since sex-linked recessive mutations of the order recorded in these experiments may occur in the control populations, it is clear that the effects produced by the irradiation of the medium are small and can be demonstrated only in relation to controls of comparable size. Although as early as 1947, Stone *et al.* (*3*) reported the incidence of mutations in *Staphylococcus aureus* cultured on ultraviolet irradiated substrate, no similar experiment in *Drosophila* appears to have been reported. Evidence of indirect effects of radiation in *Drosophila* is, however, provided by Ulrich (*4*), who found that the irradiation of cytoplasm alone of fertilized *Drosophila* eggs increased the mortality rate of the embryos and the incidence of mutations in the subsequent progeny. In view of the wide implications of such data, there is need for a more extensive and critical evaluation of the extent and pathways of indirect radiation effects (*5*; *6*).

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References and Notes

1. A. T. Natarajan and M. S. Swaminathan, *Indian J. Genet.* **18**, 220 (1958); A. T. Natarajan, *Riso Rept.* **16**, 39 (1960 → M. S. Swaminathan, V. L. Chopra, S. Bhaskaran, *Radiation Res.* **16**, 182 (1962); V. L. Chopra, A. T. Natarajan, M. S. Swaminathan, *Radiation Botany* **3**, 1 (1963).
2. T. H. Morgan, C. B. Bridges, A. H. Sturtevant, *Bibliographia Genetica* **2**, 3 (1925).
→ W. S. Stone, O. Wyss, F. Hass, *Proc. Natl. Acad. Sci. U.S.* **33**, 59 (1947).
4. H. Ulrich, *Radiation Res.* **9**, 196 (1958).
5. L. Ehrenberg, "Report of the FAO/WHO/IAEA meeting on the wholesomeness of irradiated foods. Brussels, 1961," *Food and agricultural organization*, U.N., Rome (1962), p. 166.
6. We thank Prof. H. J. Muller and Dr. C. Auerbach for their comments on the manuscript, Dr. B. P. Pal for his encouragement, and Drs. O. S. Reddi and Ruth Sävhagen for the *Drosophila* cultures.

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