amount of natural ionization in this mine with that of one mg of radium. Radiation in the mine was found to be 0.39 times as intense as that from one mg of radium when the rays were passed through a 0.156-inch lead filter.

Male flies were exposed in this mine for 140 hours and then returned to St. Louis for the breeding tests. The well-known C1B method for detecting lethal mutations in the X-chromosome was employed.

In this technique lethals are revealed in the F_2 cultures. There were 2,860 test cultures, of which seven, or 0.245 ± 0.062 per cent., produced no male flies. In the 1,308 control cultures there was one lethal mutation, or 0.076 ± 0.051 per cent. The difference between tests and controls is 0.169 ± 0.081, a difference 2.09 times its probable error.

While this difference is theoretically not statistically significant it actually may be so. It is highly probable that if the flies could have been exposed for a much longer period than the 140 hours the results would have been more striking. This was impossible in this instance as the time consumed in taking the flies to Colorado and back, together with finding a suitable location there for the test, used up a considerable portion of their life span. The results secured, however, point to much greater success when the experiment can be repeated, hatching the flies, exposing them for several weeks and breeding them for results at the mine. Or a second possibility seems equally promising, namely, from the electroscope readings in the carnotite mine exactly equivalent amounts of radiation can be duplicated in the laboratory and the time of exposure extended accordingly.

These two experiments, one in California and one in Colorado, while falling short of being statistically significant, nevertheless are consistent in that both give an actually higher rate of mutation in flies exposed to natural radiation than in the controls. The least that can be said for the results is that they strengthen definitely the plausibility of the suggestions quoted above to the effect that natural radiation may be responsible for the mutations which are the grist of the natural selection mill with the resulting evolution of new forms.[7]

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[7] We take this opportunity of expressing our appreciation to Dr. R. D. George, state geologist of Colorado, for his many helpful suggestions which led to the finding of what must be one of the most radioactive locations in Colorado; also to Mr. R. S. Blitz, of the Vanadium Corporation, who kindly permitted the tests to be made in the carnotite mine. Mr. Walter D. Claus, of the physics department of Washington University, constructed the electroscope and made the ionization tests.

THE EFFECT OF VARYING THE DURATION OF X-RAY TREATMENT UPON THE FREQUENCY OF MUTATION

IT has been shown repeatedly that X-rays produce variations. We need now to learn more about the nature of the X-ray action in producing these variations, and to obtain further evidence regarding the question whether or not such dilute amounts of radiation as are present in nature might be expected to be producing the mutations found in nature. As a step in that direction, this experiment was begun, in the early part of 1928, at the suggestion and under the supervision of Dr. H. J. Muller, to find the relation between different doses of X-rays and the resultant effect upon the individuals—more definitely, to get the relation of dosage to the frequency of sex-linked lethal mutations produced in *Drosophila melanogaster,* and to analyze the results found.

The different dosages varied only in the length of time of treatment. All other factors were kept as nearly constant as possible. Adult males having the autosomal characteristic brown-eye were collected and kept at 27° C. for at least three days before treatment. The experiment was divided into four series in each of which the flies were divided in about the same proportions among the different dosage groups. All the flies, including the controls, were handled similarly except for the time of treatment. After treatment, these males were mated to virgins containing C1B in one X-chromosome and scute vermilion forked in the other, and after seven days these P_1 flies were discarded. The F_1 bar-eyed (C1B-containing) females were mated to their brothers. Normally half the F_2 males die because of the lethal in the C1B combination. Now if a lethal had arisen by treatment of the X-chromosome of the P_1 male, there would be no F_2 males appearing. However, it is necessary to check these apparent lethals in the F_2 cultures by further breeding in order to be certain that the no-male result was due to a lethal and not to other conditions. For that purpose, the non-bar-eyed F_2 females in the apparently lethal cultures were mated to scute vermilion forked males. Only the cultures showing a lethal in these F_3 results were recorded under column three in the table given.

The results are tabulated briefly in the following table in which "dosage" refers to duration of treatment: t_1 lasted three and one half minutes; t_2, double that time, etc. As previously indicated, the total number of lethals is based on the F_3 results from the matings of the non-bar females by sc v f males. The "per cent. of observed lethals due to treatment" is found by subtracting the control lethal per cent. ($0.24 \pm .051$) from the per cent. found for each dosage. The "ideal per cent." is calculated from the

Dosage	Number fertile F_2 cultures	Total number lethals	Per cent. observed lethals due to treatment	Ideal per cent. lethals
t_{16}	435	70 ± 5.19	15.85 ± 1.19	18.07 ± .7
t_8	618	61 ± 4.57	9.63 ± .74	9.49 ± .4
t_4	1144	55 ± 4.89	4.56 ± .428	4.86 ± .2
t_2	2231	72 ± 5.63	2.99 ± .256	2.46 ± .1
t_1	4016	57 ± 5.04	1.18 ± .135	1.24 ± .05
Control	4033	10 ± 2.13		
Total	12477			

X-ray dosage: 50 KV; 10 MA; 13½ cm distance; 1 mm Al screen; time varies as indicated; t_1 approximately 285 r units.

equation based upon the law of probability: $\log q = kt$, in which q is the proportion of escaping (non-lethal) cells, t is the time of treatment and k is a constant. From this equation the mean value of k is found, and the ideal values for all the doses used are calculated from that.

The results show a significant increase in the per cent. of lethals each time the dosage is doubled, and with each dosage, the per cent. of corrected observed lethals approaches closely the ideal per cent., that is, the per cent. that would be expected based on the results as a whole, if the above equation held true. That there is a direct proportionality between the per cent. of lethals and the length of time of treatment may be seen more readily by a comparison of the t_1 values calculated from the results for each of the given doses. These values are calculated, based on the proportion of escaping cells, by using the formula previously given. The mean t_1 value is found to be $1.24 \pm .05$; t_1 values calculated from the results for each of the given doses are found to be: t_1 based on t_1 result $= 1.18 \pm .13$; t_1 based on t_2 result $= 1.506 \pm .13$; t_1 based on t_4 result $= 1.162 \pm .11$; t_1 based on t_8 result $= 1.26 \pm .10$; t_1 based on t_{16} result $= 1.074 \pm .09$.

A comparison of these values with the mean t_1 figure shows that the values based on the t_1, t_4 and t_8 doses approach closely to this mean; those of the t_2 and t_{16} vary more, the former being larger and the latter smaller. However, in each case, the difference between the observed and ideal values falls within twice its own probable error, an event to be expected once in about 5.5 trials, on the average, and hence not surprising in two of our five batches.

In the analysis of the F_3 cultures one finds some in which there is a significant decrease in crossing-over. Some of these are lethal in effect and so were recorded as lethals in the table; however, there was such a lack of crossing-over that it was impossible from our recorded results to locate the lethal or to say just what kind of lethal it was. For the present these are classed as "chromosome abnormalities" (CA). The two higher doses have a much greater per cent. of the CA lethals than do the three lower doses. We found, corrected, 4.88 per cent. of such CA's in the t_{16}, 2.204 in t_8, 0.377 in t_4, 0.403 in t_2, 0.075 in t_1 and none in the control. Calculating the t_1 value from these, the average for the two higher doses is $.292 \pm .0465$, and for the three lower doses, $.124 \pm .029$. There is, then, a difference of $.168 \pm .037$. This difference is 4.5 times its probable error, and this is apparently significant. However, it may be that some of these CA's are due to double lethal point-mutations or to lethal point-mutations combined with semi-lethal or poor-viability genes. Tests are now being made, to be reported later, by which to analyze them. The effect of dosage on the frequency of CA's can not be conclusively given till these tests are completed.

Regardless of the results for the CA's, it is important to note that the total number of lethals is directly proportional to the dosage used when the only factor varied is the duration of treatment. There is no indication of a threshold dosage below which mutations would not be produced. In so far as this work goes, it therefore indicates that the small amounts of radiation in nature may cause some or all of the natural mutations. Since the suggestion of this possibility and of the method of testing it by measurements of mutation frequency in the presence of different dosages of radiation was made by Muller,[1] Olson and Lewis[2] reported calculations indicating that in tobacco the frequency of "variations" under natural conditions bore about the same relation to their frequency following X-ray treatment as did the amount of ionization in nature to that caused by the X-raying. It is, however, uncertain whether or not gene-mutations were predominantly involved in this work and whether the types of variation in the two cases were comparable genetically. A few months later Hanson and Heys[3] obtained results in favor of the interpretation

[1] H. J. Muller, "Artificial Transmutation of the Gene," SCIENCE, 66, 84–87, 1927; "The Problem of Genic Modification," *Verhand. d. V. Int. Kong. f. Vererb.*, Berlin, 234–260, 1927; "The Production of Mutations by X-Rays," *Proc. Nat. Acad. Sci.*, 14: 714–726, 1928.

[2] A. R. Olson and G. N. Lewis, "Does Natural Ionizing Radiation Control Rate of Mutation?" *Nature*, 121: 673–674, 1928.

[3] F. B. Hanson, "An Analysis of the Effects of Different Rays of Radium in Producing Lethal Mutations in Drosophila" (Abstr.), *Anat. Rec.*, 41: 99–100, 1929; F. B. Hanson and F. M. Heys, "An Analysis of the Effects of Different Rays of Radium in Producing Lethal Mutations in Drosophila," *Am. Nat.*, 63: 201–213, 1929.

that natural radiation caused natural mutations, since their data on beta and gamma rays of radium, like the independent work on X-rays reported above, showed a proportionate relationship between dosage and mutation frequency. Stadler[4] on the basis of preliminary experiments on barley has tentatively reported an apparently similar relation between X-ray dosage and mutational effect. Still more recently Babcock and Collins[5] have reported preliminary results that point in the same direction, in that they find a difference in mutation frequency between two series of flies subjected to differing amounts of natural radiation which is 2.5 times its own probable error. The chance of occurrence of such an outcome if there were no real effect is 1 to 10. In their work, owing to the small numbers of mutations necessarily obtained with such dilute radiation, there can as yet be no question of showing a proportionality between mutation frequency and radiation. However, the concurrence of the evidence from all the above sources is noteworthy.

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OVARIAN CHANGES DURING PREGNANCY IN THE RAT

IN an earlier issue of SCIENCE, Nelson[1] reports the recurrence of oestrus cycles four days in length with copulation taking place at three of the oestrus periods in a pregnant white rat. Two instances of copulation during pregnancy had been reported by Long and Evans,[2] though no evidences of the normal oestrus cycle were noted. In the later report no attempt was made to determine the occurrence of ovulation.

In a preliminary communication[3] we have noted the occurrence of ovogenesis during adult life in the mammalia as a rhythmical production which coincides with the rhythm of the oestrus cycle. In each oestrus cycle a new crop of follicles is formed and a few grow to maturity. At each oestrus period ovulation takes place, and all the other follicles degenerate within a short time thereafter. We have found that this cycle of ovogenesis in the rat is not interrupted by pregnancy, but continues throughout with the usual four or five-day periods. At the end of each period ripe follicles are present with many smaller follicles showing all stages of degeneration. With the beginning of the new cycle at the fifth, the tenth, the fourteenth and the eighteenth days, or thereabouts, newly formed corpora are found, some of which contain a segmenting ovum. These corpora are seldom more than a third of the size of the corpora of pregnancy, and are often much less than that. The number of large follicles produced at each cycle varied from two to twelve in the seventy-six rats that were examined at all stages of pregnancy from the third day to full term. The number of small corpora showed about the same range of variation though occasionally small follicles were luteinized.

No changes in the uterus corresponding to those of the non-pregnant oestrus cycle were observed. The living animals were not examined by means of the vaginal smear method. This has been done in hundreds of pregnant rats in our laboratory, however, without detecting the typical oestrus changes, though it has been noted for many years that at day five the smear loses its typical appearance, resembling a prooestrus smear, especially in the reduction in leucocytes, though it is not followed by cornified cells. In their studies of the uterus of pregnant rats, Long and Evans[2] found no evidence of cyclical changes. No eggs were found in the tubes, though comparatively few of the corpora showed a retained egg.

Twenty-three of these rats were tested for oestrus behavior by being placed in a cage with an active male on the fifth day. No evidences of oestrus were observed and copulation did not take place in a single instance, as shown by the absence of plug and sperm when examined on the following day.

There is no evidence in these animals that the corpora lutea of pregnancy have any effect on the production of a normal number of follicles and their maturation. The evidence also shows that the cervical stimulation of copulation at the beginning of pregnancy did not result in the delayed production of mature follicles, as these were present at the fifth day in eight rats. It also indicates that the presence of fairly large follicles in the ovary is not sufficient to produce oestrus changes in the uterus or oestrus behavior in the animal, although corpora lutea are actually developed from such follicles. Cyclical changes thus occur in the ovary during pregnancy, a condition which has hitherto been supposed to suspend those changes.

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[4] L. J. Stadler, "The Rate of Induced Mutations in Relation to Dormancy, Temperature and Dosage" (Abstr.), *Anat. Rec.*, 41: 97, 1928.

[5] E. B. Babcock and J. L. Collins, "Does Natural Ionizing Radiation Control Rate of Mutation?" *Proc. Nat. Acad. Sci.*, 15: 623–628, 1929.

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[2] J. A. Long and H. M. Evans: "The Oestrus Cycle in the Rat and Its Associated Phenomena," Mem. Univ. Calif., Vol. 6, 1922.

[3] O. Swezy and H. M. Evans: "Ovogenesis in the Mammalia," *Proc. Exp. Biol. and Med.*, Vol. 27, 1929.