THE INTERPRETATION OF CARTER'S RESULTS ON INDUCTION OF RECESSIVE LETHALS IN MICE

By J. B. S. HALDANE
Indian Statistical Institute, Calcutta-35

Carter (1959) using a method suggested by Haldane (1956) bred from 158 pairs of irradiated mice and as many controls. He concluded that two recessive lethals had appeared by mutation among the controls, and only one among the irradiated mice. I give reasons why this conclusion seems to me questionable. It can be argued that three of Carter's irradiated pairs showed evidence that each was heterozygous for a recessive lethal. If this is so his further conclusions require considerable modification.

Carter's method was this. A stock of mice homozygous for six recessive markers (counting **d** and **se** as one) was irradiated. The total dose was 600 r to the father and both grandfathers of the mice whose gametes were tested. It is not stated whether X or gamma rays were used, but the dose appears to have been fairly acute, and breeding began after the sterility following it had ceased. The progeny of an irradiated father and two irradiated grandfathers were mated to wild-type mice, and from each mating one F_1 pair was mated. 158 pairs raised four litters each. 11 of these F_2 contained so few of one or other of the six recessives as to "qualify for further investigation". This took two forms. Further F_2 litters were raised, where possible. And the F_1 father was mated to daughters of "wild" phenotype (at the locus under investigation) previously shown to be heterozygous by a test mating. Full details are given in Carter's Tables 3, 4, and 5.

Let us consider pair 126. The first four litters included only 3 **a**/**a** (non-agouti) mice out of 51. A fifth litter of 13 containing no **a**/**a** confirmed the hypothesis of a lethal linked with **a**. The F_1 mother then died. A number (not stated) of "wild-type" i.e. +/+ or +/**a** daughters were tested by mating with **a**/**a** males. Four were found to be heterozygous and mated with their father. They produced 8 **a**/**a** out of 43, 8 out of 48, 13 out of 46, and 6 out of 12. I now quote Carter. "In pair 126 the female died after raising her fifth litter; her great fertility (64 young classified in 5 litters) argues against the presence of a lethal, but if one were present, its recombination with **a** would be estimated to be 7·3 per cent. In that event the expected frequency of **aa** homozygotes, upon back-crossing to the F_1 male his + **a** daughters, would be 9·8 per cent, or 14·6 among 149 classified young; the observed number, 35, is greatly in excess of this. It must be concluded that there was no linked lethal present."

I believe that this argument is incorrect and misleading for several reasons. First, in such a case it is incorrect to choose, in order to test a hypothesis, the value of an unknown parameter given by a part only of the numerical data.

Let us ask two questions.

(1) What is the frequency with which a pair would give 3 or fewer recessives out of 64 in the absence of a linked lethal, 16 recessives being expected ?

(2) What is the frequency with which one set of matings would give 3 recessives out of 64 and another 35 out of 149 when the same frequency was expected in each ?

The answer to the first question is

$$P = 4^{-64} \times 3^{61}(3^3 + 3^3 \cdot 63 + \tfrac{1}{2} \cdot 3 \cdot 63 \cdot 62 + \tfrac{1}{6} \cdot 63 \cdot 62 \cdot 61)$$
$$= 4^{-64} \times 3^{63} \times 13954$$
$$= 4{\cdot}694 \times 10^{-5}.$$

The answer to the second is approximately found from the 2×2 table

61	3
114	35

which gives $\chi^2_1 = 10{\cdot}78$, $P = 1{\cdot}03 \times 10^{-3}$.

Thus even if we suppose that all the tested daughters had the same genotype as their mother, it seems that we should be justified in deciding in favour of the less unlikely hypothesis, namely that a linked lethal was present. It can easily be seen that the estimate of the recombination frequency is about 32%. However if Carter's argument were correct, we should be forced to choose between two hypotheses both of which are very improbable.

We have now to consider Carter's second assumption. This is that if we calculate the expected number of recessives in the progeny of a group of families produced by back-crossing to daughters, we can then treat this estimate as if it were obtained on the basis of Mendelian expectation. This is not so. The daughters may be of three different genotypes, and if, as in this case, four daughters are used, there are 81 possible expectations, and it is not a simple matter to calculate the standard error of their weighted mean. To explain the calculations which follow, it will be necessary to repeat some of the argument of Haldane (1956). I assume a recessive marker a linked with a recessive lethal l, the frequency of recombination being p. I have shown that the differences in recombination frequency between the two sexes may be neglected without serious loss of accuracy. In a F_2 or other family from $\frac{+\ +}{a\ l} \times \frac{+\ +}{a\ l}$, we expect the following frequencies of genotypes:

+ +/+ +	$\frac{1}{4}(1-p)^2$	
+ +/+ l	$\frac{1}{2}p(1-p)$	
+ +/a l	$\frac{1}{2}(1-p)^2$	$\frac{1}{2}(1-p+p^2)$
+ +/a +	$\frac{1}{2}p(1-p)$	
a +/+ l	$\frac{1}{4}p^2$	
a +/a +	$\frac{1}{4}p^2$	$\frac{1}{2}p(2-p)$
a +/a l	$\frac{1}{2}p(1-p)$	

The expected frequency of recessives is $x = \frac{1}{4}p(2-p)$. The three genotypes of +/a daughters are all detected with equal frequency by matings with a +/a + males. Their frequencies, among +/a daughters are:

$$\frac{(1-p)^2}{1-p+p^2} + +/a\ l,\ \frac{p(1-p)}{1-p+p^2} + +/a\ +,\ \text{and}\ \frac{p^2}{1-p+p^2}\ a\ +/+\ l.$$

Mated with their father, the expected frequencies of recessive offspring are $\frac{1}{2}p(2-p)$, $\frac{1}{4}$, and $\frac{1}{2}(1-p+p^2)$. For example if $p=0{\cdot}25$, which we shall see is a plausible value, we should expect only $\frac{9}{32}$ of the daughters to be $+\ +/a\ 1$ like their mother. And the probability that all four daughters mated to the father were $+\ +/a\ 1$ is only ·2297. Thus Carter's results become a great deal more explicable, since one or two of the tested daughters were probably $+\ +/a\ +$.

A rigorous calculation, either of the likelihood of a value of p in a given neighbourhood, or of the probability of getting a worse fit to expectation than the observed, is extremely laborious. For each value of p it would be necessary to compute probabilities for each of the 81 different sets of genotypes to which the four daughters might belong. I shall merely show that some values of p are quite likely, on the evidence given by Carter. This is all that is usually done in a F_2 analysis.

Let $p=·25$, and suppose, first, that one daughter was $\frac{+\ +}{a\ +}$ and three were $\frac{+\ +}{a\ 1}$. This would occur in 30·6% of all cases. Further suppose that this daughter was the one which produced 6 recessives out of 12. We have the results of Table 1. $\chi^2_6=16{\cdot}66$, $P=·0054$. If however throughout the expectation is one quarter recessives we have the results of Table 2. $\chi^2_6=20{\cdot}93$, $P=·00084$. Let us now suppose that two daughters

TABLE 1

Mother of family	Total	Recessives	Expectation	χ^2
F_1 female	64	3	9·333	5·031
F_2 ,, 1	43	8	6·271	0·558
,, ,, 2	48	8	7	0·167
,, ,, 3	46	13	6·708	6·908
,, ,, 4	12	6	3	4·000
Total	213	38	32·312	16·664

χ^2 table for pair 126, assuming p=·25, and the daughter 4 $\frac{+\ +}{a\ +}$, the other 3 $\frac{+\ +}{a\ 1}$.

TABLE 2

Mother of family	Total	Recessives	Expectation	χ^2
F_1 female	64	3	16	14·083
F_2 ,, 1	43	8	10·75	0·937
,, ,, 2	48	8	12	1·778
,, ,, 3	46	13	11·5	0·130
,, ,, 4	12	6	3	4·000
Total	213	38	63·25	20·928

χ^2 table for pair 126, assuming no lethal present.

were $\frac{+\ +}{a\ +}$ and two $\frac{+\ +}{a\ l}$. This would be so in 15·3% of all cases. Further suppose that the two $\frac{+\ +}{a\ +}$ daughters were the mothers of 46 and 12 offspring respectively. In Table 2 we have to substitute 0·130 for 6·908 in the χ^2 column. χ^2_5 is reduced to 9·886. P=·074, which is not significantly low. A similar calculation for $p=·20$ gives $\chi^2_5=11{\cdot}955$. These values would be slightly lowered if the mother (4) of the family of 12 were $\frac{a\ +}{+\ l}$, in which case χ^2 is reduced by 1·964. I conclude that there is no serious reason to doubt that the members of pair 126 were heterozygous for the same lethal, probably distant 20-25 cM from the locus of a.

Similarly let us consider pair 107. The recessive marker with which linkage was suspected is c^{ch}. I have used the symbol c for convenience. The numbers are unfortunately small. In Table 3 I give the expectations on the hypothesis that $p=·25$,

TABLE 3

Mother of family	Total	Recessives	Expectation	χ^2
F_1 female	42	3	6·125	1·870
F_2 „ 3	31	10	7·75	0·871
„ „ 1	38	11	9·5	0·316
„ „ 4	30	7	4·375	0·820
„ „ 5	43	7	6·271	0·099
„ „ 2	38	4	5·542	0·502
Total	222	42	39·563	4·478

χ^2 table for pair 107, assuming p=·25, and daughters 3 and 1 to be $\frac{+\ +}{a\ +}$, the other three being $\frac{+\ +}{a\ l}$.

and 3 daughters were $\frac{+\ +}{c\ l}$ while 2 were $\frac{+\ +}{c\ +}$. This is the most probable distribution, since the frequencies of $\frac{+\ +}{c\ l}$, $\frac{+\ +}{c\ +}$, and $\frac{c\ +}{+\ l}$ daughters are $\frac{6}{16}$, $\frac{4}{16}$, and $\frac{1}{16}$. I further assume that daughters 3 and 1 were $\frac{+\ +}{c\ +}$. $\chi^2_6=4{\cdot}478$, P=·61. The fit is excellent, Table 4 shows that with $p=·5$ (no lethal) $\chi^2_6=12{\cdot}60$, P=·050. No doubt a better fit could be obtained by using a different value of p, and assuming that daughter 3 was $\frac{c\ +}{+\ l}$, as she may have been. Moreover the value ·05 is too high, for it would probably be better not to consider daughters 3 and 1. We should then

have $\chi^2_4 = 11{\cdot}418$, $P = {\cdot}025$. We cannot come to a very firm conclusion, given the small sizes of the families. But there is no ground for rejecting the hypothesis of a linked lethal, as Carter claims. And as a rule for decision has been laid down, this pair should, I think, be regarded as heterozygous for the same lethal.

The remaining suspected progenies from irradiated ancestries were regarded as false clues because in the fifth and later litters recessives appeared in such numbers as to lead to rejection on the criteria given in Carter's Table 1.

I conclude then, that among 158 pairs from unirradiated ancestors there were two lethals; among 158 from irradiated ancestors, there were two lethals (in pairs 55 and

TABLE 4

Mother of family	Total	Recessives	Expectation	χ^2
F_1 female	42	3	10·5	7·143
F_2 " 3	31	10	7·75	0·871
" " 1	38	11	9·5	0·316
" " 4	30	7	7·5	0·044
" " 5	43	7	10·75	1·047
" " 2	38	4	9·5	3·184
Total	222	42	55·5	12·605

χ^2 table for pair 107, assuming no lethal present.

126) with very high probability and one more (in pair 107) with a moderately high probability, which must however be taken as present according to the criteria adopted, and therefore the length of chromosome scanned.

The length scanned in the irradiated series is only very slightly less than in the control series (see Carter's Table 6). The number of lethals found according to my argument, namely 3, is of course not significantly greater than 2. It is quite consistent with the expectation calculated by Carter from Russell's data, namely 2·3, the control value being unexpectedly high. On Haldane's (1956) "guess" of one recessive lethal mutation per gamete per 300 r, confirmed by Carter (1957) we should have expected about 14 lethals. This figure is therefore almost certainly too high. But a figure of 1 per 600 r is quite possible.

One of the most surprising features of Carter's work is the discovery of two recessive lethals in the controls. Carter states that his multiple recessive (PCA) stock was maintained with the minimum amount of inbreeding. This may perhaps be undesirable. We must reckon with the possibility that some lethal genes increase fitness in heterozygotes, and will therefore spread in outbred populations.

If it is considered that my criticism of Carter's conclusions is valid, I must accept a fair share of the blame, as I only dealt in a rather cursory manner with the

confirmation of suspected lethals. However it is to be noted that Carter did not use the principal method which I suggested, namely the use of the recessive mice appearing in F_2 to build up new stocks heterozygous for the lethal and the marker gene.

I do not know whether research on these lines is being continued at Harwell. According to Sugahara, Tutikawa, and Tanaka (1959) it is being continued at the National Institute of Genetics, Misima, Japan. It is, I think, clear from my discussion that each lethal believed to have been detected should be very thoroughly investigated. This is in any case desirable, since it is important to know at what stage in the life cycle a lethal acts; and if any extrapolation is made to man, this is very important (Haldane 1956).

SUMMARY

An analysis of Carter's data on the induction by X-rays of autosomal recessive genes in mice leads to the conclusion that he almost certainly obtained two, and very probably three such lethals in his irradiated stock, whereas he only claims to have obtained one.

REFERENCES

CARTER, T. C. (1957). Recessive lethal mutation induced in the mouse by chronic gamma radiation. *Proc. Roy. Soc. B*, **147**, 402-411.

CARTER, T. C. (1959). A pilot experiment with mice, using Haldane's method for detecting induced autosomal recessive lethal genes, *J. Genet.*, **56**, 353-362.

HALDANE, J. B. S. (1956). The detection of autosomal lethals in mice induced by mutagenic agents. *J. Genet.*, **54**, 327-342.

RUSSELL, W. L. (1959). Genetic effects of radiation in mammals. Radiation Biology 825-859 (New York).

SUGAHARA, T., TUTIKAWA, K., AND TANAKA, T. (1959). Studies on mutation rates after chronic irradiation in mice. A preliminary report on the sex ratio. *Ann. Rep. Nat. Inst. Genetics, Japan*, **9**, 102-104.