x-rays in the production of mutations of the two categories. Using a physical estimate of the number of ions per cc., the uncertainty of which would not affect the relative results, neutron-produced ions were found to be about 0.75 times as efficient as x-ray-produced ions in the induction of recessive sex-linked lethal mutations (which is in reasonable agreement with the results of Timofeeff-Ressovsky and Zimmer,[3] considering the differences in ionization-measuring instruments), and about 1.5 times as efficient in the production of dominant lethals, where doses of x-rays and neutrons are applied which result in the death of 50% of the F_1 eggs.

It may be significant that the efficiency of neutron-produced ions in the induction of gross (genetically detected) translocations appears to be intermediate, compared to x-rays, to their efficiencies in the production of recessive and dominant lethals. The factor obtained (approximately 1.25) applies for a dose equivalent to 2500 r units of x-rays.

The data obtained are not critical for determining whether there may be some differences in the general dosage-aberration relationships for neutrons, as compared to x-rays; such a difference has been reported by Giles[4] for the case of exchange breaks in *Tradescantia* microspores.

[1] *Carnegie Inst. of Washington, Yr. Bk.*, No. 37, 40–47 (1938).
[2] *Amer. Nat.*, **68**, 166–167 (1934).
[3] *Naturwiss.*, **26**, 21–22, 362–365 (1938).
[4] *Proc. Nat. Acad. Sci.*, **26**, 567–575 (1940).

LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS) IV

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A new mutation of the rat, "wobbly," was first observed in September, 1933, in the laboratory of Professor Amy L. Daniels at the University of Iowa, in the course of an experiment in which the effects of variation in the manganese content of the diet were being studied. This mutation is a simple recessive in inheritance and is characterized by a peculiarity of locomotion of affected individuals first observable at about the time the eyes open at the age of 14 days or earlier, locomotion being jerky instead of the normal steady gliding movement of the young when taken from the nest and placed on a table. The young wobbly individual may stand in a hesitating attitude with head and particularly tail elevated, before it begins to crawl. Then it progresses a step at a time with a slight jerk of the body first forward then backward as each step is taken. The contrast is

very striking to the steady, smooth locomotion of normal animals, with head and tail horizontal. The peculiar behavior of the wobbly rats was at first thought to be an indication of manganese poisoning but this explanation was abandoned when repetition of the experiment failed to reproduce the wobbly symptoms.

Dr. Daniels states that the animals used in the first experiment were descendants of the Wisconsin stock which had been inbred in her laboratories here in Iowa and in Wisconsin since 1914. Inquiry of Professor Steenbock regarding the development of any such abnormality among his animals which were of the same original stock threw no light on the subject. None of his rats had ever shown such symptoms. Dr. Ingram, the neurotomist who saw these animals, thought the condition was the result of an infection of the inner ear. Histologic sections, however, showed no abnormality. This is a finding similar to that made by several investigators in the case of the Japanese waltzing mouse.

In repeating the experiment new uncontaminated animals were fed during four generations various levels of manganese (from 1.5 mg. to 30 mg. per rat per day) as manganese sulphate. The manganese sulphate in solution was added to a small amount of milk and fed apart from the stock ration, each animal thus consuming his allotted portion. Neither during the year nor subsequently have there appeared any rats with these queer symptoms.

Having failed to reproduce the wobbly symptoms by manganese and manganese-fluorine diets, Dr. Daniels made a reëxamination of her records and found that a certain number of rats from the stock colony had been used in a comparative study of bone development in rats and human infants, in the course of which the rats had been subjected to x-rays, and later restored to the stock colony. It is possible but not certain that one or more x-rayed individuals may have been among the ancestors of wobblies, but even if this were true, it is doubtful whether the x-raying can reasonably be regarded as the causative agent, since spontaneous mutations affecting nervous behavior (waltzing in rats, mice, and guinea-pigs, "shaker" behavior in mice) have occurred without x-ray treatment.

When Dr. Daniels came to the conclusion that the wobbly state was due to a genetic mutation, however caused, she offered generously to share her material with the co-authors for a study of its linkage relations with other known genes, of which they possessed a nearly complete assortment. Accordingly wobbly stocks were established in Berkeley and Philadelphia and a comprehensive program of linkage tests was outlined.

At that time two linkage systems had been established for the rat, as follows. Chromosome I was known to contain five mutant genes, in the order *l c r p w*. Chromosome II was known to contain two mutant genes, *Cu* and *b*, but while this investigation was in progress a third mutant gene

an was found to lie in Chromosome II, the order of the three genes being *Cu an b*. Seven other mutant genes had been shown to be independent of genes in Chromosomes I and II and of one another, thus being markers of as many independent chromosomes. They are genes *a*, Cu_2, *d*, *h*, *hr*, *j* and *k*.

To test the linkage relations of wobbly with one or more genes from each of the linkage systems and with each of the seven independent genes, the following crosses were made:

I. 1. Wobbly (*wo*) × pink-eye (*p*);
   1ª. Wobbly (*wo*) × albino (*c*);

II. 2. Wobbly (*wo*) × anemia (*an*);
   2ª. Wobbly (*wo*) × curly (*Cu*);

3. Wobbly (*wo*) × agouti (*A*);
4. Wobbly (*wo*) × curly$_2$ (Cu_2);
5. Wobbly (*wo*) × dilution (*d*);
6. Wobbly (*wo*) × hooded (*h*);
7. Wobbly (*wo*) × hairless (*hr*);
8. Wobbly (*wo*) × jaundiced (*j*);
9. Wobbly (*wo*) × kinky (*k*).

Some of these crosses were made in combination, as, for example, 3 and 6, the original wobbly stock being homozygous for black hooded (*aa hh*). All data concerning the same gene from whatever cross obtained have been combined in a single total, as recorded in the tables.

Seven of the test crosses were made in Berkeley and four (1ª, 2ª, 7 and 9) were made in Philadelphia. From six of the eleven crosses F_1 animals were back-crossed to double recessive individuals, from which sort of matings approximately equal numbers of crossover and non-crossover combinations are expected, if no linkage exists. Such a result was actually obtained, as shown by the data included in table 1.

TABLE 1

BACK-CROSS DATA ON THE LINKAGE RELATION OF WOBBLY TO SIX OTHER MUTANT GENES

CROSS	GENE	NON-CROSSOVERS	CROSS-OVERS	DEVIATION	P.E.	DEV./P.E.	OBSERVER
1	*p*	93	97	2.0	4.6	0.43	Castle
2ª	*Cu*	24	25	0.5	2.3	0.02	King
3	*a*	45	37	4.0	3.0	1.30	Castle
4	Cu_2	70	70	0	4.0	0	Castle
5	*d*	103	107	2.0	4.9	0.41	Castle
6	*h*	211	188	11.5	6.7	1.70	Castle

For the four remaining crosses (2, 7, 8 and 9) backcrosses of the ordinary type were not possible since double recessive individuals either could not be obtained or were very feeble because of the lethal or enfeebling character of

the combination. Accordingly a method outlined by Castle in 1939 was followed. F_1 was mated to animals carrying neither of the two genes involved in the cross. The progeny of such matings were tested individually for presence of each of the two genes contributed to F_1 in the repulsion relationship. The tests were made by mating the individual to be tested to an ordinary F_1 individual. A litter of six or more young was considered a conclusive test. The animals tested fall into four classes, viz. (1) carriers of both mutant genes, (2) carriers of neither, (3) carriers of one gene only and (4) carriers of the other gene only. Classes (1) and (2) would have received crossover combinations from the F_1 parent; classes (3) and (4) would have received non-crossover (repulsion) combinations from the F_1 parent. From table 2 it will be seen that in crosses 2, 8 and 9, no significant

TABLE 2

DATA ON THE LINKAGE RELATION OF WOBBLY TO FOUR OTHER MUTANT GENES, OBTAINED BY THE "LETHAL TEST" METHOD

CROSS	GENE	NON-CROSSOVERS	CROSS-OVERS	DEVIATION	P.E.	DEV./P.E.	OBSERVER
2	*an*	82	79	1.5	4.3	0.35	Castle
7	*hr*	71	48	11.5	3.68	3.12	King
8	*j*	72	71	0.5	4.0	0.12	Castle
9	*k*	65	66	0.5	3.8	0.13	King

difference is found between the totals for crossover and non-crossover combinations, respectively, but in cross 7 a significant difference is found, non-crossovers being in excess, showing repulsion between wobbly and hairless, which establishes their location in the same chromosome (which we shall designate Chromosome III) with an indicated loose linkage between the two genes of 40.33 ± 3.09 per cent, which is very similar in amount to the linkage in Chromosome II, between genes *Cu* and *b*, which was found by King and Castle (1935) to be 40.48 ± 1.35.

In testing for linkage between hairless and wobbly (cross 7) by the lethal test method, Dr. King mated 119 outcross individuals to F_1 animals. Of these

Non-crossovers { 32 produced normal and wobbly young;
39 produced normal and hairless young;
Crossovers { 20 produced normal and both wobbly and hairless young;
28 produced normal but neither wobbly nor hairless young.

The odds are 27 to one against the occurrence of so great an inequality as this between crossover and non-crossover classes by chance alone.

Supplementing cross 1, and confirming the finding from the cross with pink-eye that wobbly does not lie in Chromosome I, a cross (1^a) was made

by King between wobbly and albino (*c*). An F_2 population of 186 young consisted of the following classes:

	C Wo	*C wo*	*c Wo*	*c wo*
Observed	87	41	45	13
Expected	104.6	34.9	34.9	11.6

No indication of linkage is given by this population, since the critical recombination class *c wo* which could arise only from crossover gametes is even slightly greater than expected.

Summary—The mutant gene "wobbly" discovered by Daniels has been shown by linkage tests made by King and Castle to lie in the same chromosome (III) as hairless, with a crossover percentage of 40.3 ± 3.1.

[1] King, Helen Dean, and Castle, W. E., "Linkage Studies of the Rat," *Proc. Nat. Acad. Sci.*, **21**, 390–399 (1935).

[2] King, Helen Dean, and Castle, W. E., "Linkage Studies of the Rat, II," *Ibid.*, **23**, 56–60 (1937).

[3] Castle, W. E., and King, Helen Dean, "Linkage Studies of the Rat, III," *Ibid.*, **26**, 578–580 (1940).

[4] Castle, W. E., "On a Method for Testing for Linkage between Lethal Genes," *Ibid.*, **25**, 593–594 (1939).

[5] Grüneberg, H., "Linkage Relations of a New Lethal Gene in the Rat," *Genetics*, **24**, 732–746 (1939).

THE EFFECT OF ADULT BODY COLOR MUTATIONS UPON THE LARVA OF DROSOPHILA MELANOGASTER

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In an organism widely used, as is *Drosophila melanogaster*, for genetical, cytological and embryological experimentation, the effects brought about during development by those mutations which alter the adult phenotype are of importance from both the theoretical and the practical standpoints. From the theoretical point of view, the genetic factors whose effects are recognizable during embryogeny offer rich material for the study of the mechanism of gene action, since observations upon the early action of a gene may provide information which is important in understanding its effect upon the adult. From a utilitarian standpoint, mutants which can be identified during the larval period are useful in classifying the larvae required for salivary chromosome preparations, for transplantation experi-