GENETIC LINKAGE IN THE RABBIT

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The first discovered case of linkage in the rabbit was found by Castle (1924) to exist between albinism and brown coat pigmentation. Later it was shown by Pease (1927) that yellow fat also is linked with albinism. Castle (1933, 1936) then showed that yellow fat is also linked with brown, as was to be expected, the order of the three genes and their map distances apart being *c* 14.4 *y* 28.4 *b*. These are the values given by F_1 females, those found for males being lower as in mice and rats, as follows: *c* 8.2 *y* 26.6 *b*. Interference due to double crossing-over was found to occur in this chromosome, as in *Drosophila* chromosomes, reducing the crossovers between *c* and *b* from a known 42.8 per cent to an apparent 38.3 per cent in females, and from a known 34.8 per cent to an apparent 25.7 per cent in males. The chromosome, in which the three genes, albinism, yellow fat and brown pigmentation lie, may be called Chromosome I of the rabbit.

A second linkage system was shown by Castle (1926) to contain the genes for English and Dutch patterns of white spotting as well as the gene for long (angora) hair. English and Dutch were shown to be very closely linked with each other with less than one per cent of directly observed crossing-over between them, but since Dutch showed a greater frequency of crossing-over with angora than English did, the conclusion was reached that Dutch was more remote from angora, and the order of the genes and their map distances apart were estimated thus:

du 1.19 *En* 13.07 *l*

The chromosome which bears these three genes may be called Chromosome II of the rabbit.

A third linkage system was shown to exist in the rabbit by Castle and Nachtsheim (1933) between two genes for short (rex) hair (r_1 and r_2). The crossover percentage between them was found by Castle (1936) to be 17.2 ± 0.4. They serve as markers of rabbit Chromosome III.

A fourth linkage system was found by Sawin (1934) to involve the gene for agouti hair pattern and a modification of agouti in which the agouti band is unusually wide. The crossover percentage between agouti (*A*) and wide band (*w*) was found to be about 30. To this fourth linkage group may now be added a third mutant gene first described by Greene, Hu and Brown (1934) and designated by them dwarf.* In homozygous dwarf individuals the growth rate is greatly reduced so that the birth weight is less than half that of normal sibs. The dwarfs die of starvation within a few days after

birth because of inability to nurse. Even in heterozygous dwarfs birth weight is reduced by about one third and the ears are shortened, as they are regularly in rabbits of small body size. The gene is thus an incompletely dominant lethal, or semi-lethal. For this reason the usual method of linkage analysis, backcross of the double heterozygote to a double recessive, is impracticable. It is necessary to use, in place of double recessives, animals which are heterozygous for dwarf, though homozygous for the other gene to be tested. Consequently the expected inheritance ratio becomes 3:1:3:1, instead of the usual 1:1:1:1, if no linkage is involved.

Tests of this character have shown no significant departure from expectation in the case of genes *c* (albino) and *En* (English spotting), markers of Chromosomes I and II, respectively. But in the case of the agouti gene (*A*) unmistakable evidence of linkage is obtained. In a backcross population of 304, there were 91 dwarfs, or 29.9 per cent where 25 per cent are expected. There is accordingly no deficiency of dwarfs but rather an excess, which shows that dwarfs are fully viable up to the time of birth.

Since the non-dwarf classes of the backcross population would include both individuals which were heterozygous for dwarf and those which were free from the dwarf gene, but were indistinguishable without a breeding test, it seems best to disregard them and base the calculation of the crossover percentage solely on the dwarf population in which crossover and non-crossover classes are clearly separable. Of the 91 dwarfs, 11 represent recombinations derived from a crossover gamete furnished by the doubly heterozygous parent, whereas the remaining 80 represent non-crossovers. The indicated crossover percentage is thus 12.1 ± 2.3.

The backcross population included two lots, one of which involved an initial coupling cross, the other a repulsion cross. The raw data supplied by these are as follows:

	DwA	*Dw a*	*dw A*	*dw a*	TOTAL
Coupling	84	66	9	53	212
Repulsion	29	34	27	2	92
					304

Dr. E. L. Green has kindly studied these data by the method of maximum likelihood as described by Mather (1938). This method of treatment makes use of the non-dwarf as well as the dwarf classes and leads to the conclusion that the crossover percentage is "most likely" 14.7 ± 2.6, no allowance, however, being made for a possible influence of the sex of the F_1 parent. This figure (14.7) is not very different from that derived from the dwarfs alone (12.1) so we may safely conclude that the crossover percentage is close to 12–15 per cent.

Since, in linkage group IV, genes *A* and *w* are separated by some 30 map

distance units, and *dw* is only 12–15 units from *A*, it follows that *dw* must lie between *A* and *w*, unless it lies on the opposite side of *A* from *w*, in which case the map distance between *dw* and *w* would be about 42–45 units, a not impossible but less probable situation. To decide between these alternatives an investigation of the crossover percentage between *dw* and *w* is necessary and is now in progress.

The existence of linkage in a fifth chromosome (V) between furless and brachydactyl was indicated by studies of J. C. Wightman in Sawin's laboratory. Wightman in an unpublished master's thesis reported negative results from backcross matings involving tests for linkage between brachydactyl and four other mutant genes as follows:

	NON-CROSSOVERS	CROSSOVERS	DEV./P.E.
with English (*En*)	149	154	0.31
with dilution (*d*)	54	40	2.11
with agouti (*A*)	34	35	0.17
with albinism (*c*)	10	11	0.32

In the case of a fifth gene, furless (*f*) he was unable to make backcross matings, but observed in an F_2 of 62 animals the occurrence of only one double recessive combination whereas four would be expected if the two genes lie in different chromosomes. Since at that time facilities were not available for a more extensive test at Brown University, Sawin reported the case to Castle who undertook its investigation by the so-called lethal test method.

Brachydactyl is a character of fair viability, discovered and described by Greene (1935) to whom we are indebted for a foundaton stock of this mutation as well as of dwarf. Its embryology has been studied by Inman (1941). Homozygous furless animals are of low vitality and usually sterile, so it was necessary to employ rabbits heterozygous for furless in the initial cross with brachydactyl. For this cross two F_1 males shown by breeding tests to be carriers of furless (as well as of brachydactyl) were now outcrossed to rabbits carrying neither recessive gene. Four classes of young should result from this cross, all numerically equal, if no linkage exists between furless and brachydactyl, viz., (1) *Ff Br Br*, (2) *FF Br br*, (3) *Ff Br br* and (4) *FF Br Br*. All four classes would be alike (normal) in appearance, differing only in the recessive genes which they carried. It was necessary therefore by a breeding test in the case of each individual to ascertain whether or not it carried either recessive gene. This was accomplished by mating each animal to an F_1 individual known to be a carrier of both genes. A litter of 6 or more young was considered a satisfactory test. Class (1) and class (2) animals would have received a *non-crossover* gamete from the F_1 parent used in the outcross, but class (3) and class (4) animals would have received *crossover* (recombination) gametes. If the

sum of the class (1) and class (2) individuals were significantly greater than the sum of classes (3) and (4), linkage would be indicated. A test has been made of 99 outcross animals distributed in the four classes thus:

(1)	(2)	(3)	(4)
34	37	13	15

The sum of (1) and (2), the non-crossover classes, is 71; the sum of (3) and (4), the crossover classes, is 28, which is 28.3 ± 3.0 per cent of the population, 99. There can accordingly be no doubt that furless and brachydactyl are borne in the same chromosome at an indicated map distance of 28.3 units. They thus constitute linkage group V of the rabbit. It should be noted that this estimate is based solely on the recombination occurring among the gametes produced by F_1 males. In F_1 females the crossover percentage would probably be found to be somewhat greater, possibly 30 or more.

Summary.—Five linkage groups have now been demonstrated in the rabbit. These are genetic markers of 5 of the 22 chromosome pairs. The linkage maps may be diagrammed thus:

I	*c*	*y*	*b*
	0	14.4	42.8
II	*du*	*En*	*l*
	0	1.2	14.3
III	r_1		r_2
	0		17.2
IV	*A*	*dw*	*w*
	0	14.7	30.1
V	*f*		*br*
	0		28.3

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FORMATION AND REDUCTION OF INDOPHENOL BLUE IN DEVELOPMENT OF AN ECHINODERM

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The indophenol blue reaction, often called the Nadi reaction, is an oxidation of para-aminodimethylaniline (dimethylparaphenylenediamine) and α-naphthol catalyzed by an intracellular oxidase, "indophenol oxidase," with resulting formation of indophenol blue. Indophenol oxidase is generally believed to play an important rôle in oxidative metabolism and, according to recent work, it is a part of the cytochrome–cytochrome–oxidase system.[1] The reaction has ordinarily been used as an indicator of presence or localization of the oxidase but the concentrations of agents commonly used are highly toxic to living tissues and organisms and kill rapidly. For example, one formula for the Nadi reagent is: α-naphthol 0.144%, para-aminodimethylaniline 0.108%, Na_2CO_3 0.25%. In recent work on echinoderm development Ranzi[2] mixed equal volumes of 1% solutions of the reagents, with KOH added to the α-naphthol. The mixture was added to a drop of sea water containing the echinoderm eggs or embryos in amounts not stated by the author. Concentrations of this order are immediately or very rapidly lethal to developmental stages of echinoderms and, as will appear, show nothing of the conditions in the living animals.

Use of the Method with Living Material.—In experiments with ciliate protozoa, oligochetes and echinoderm and ascidian developmental stages it has been found possible by using the reagents in extremely low concentrations to obtain the reaction in living, normally active organisms. With this procedure intracellular indophenol formation becomes a delicate indicator of certain regional differentials in physiological condition in living embryos and larval stages and their changes in the course of development.

Moreover, with slight decrease of oxygen content of the fluid containing the animals reduction of the intracellular indophenol blue to a colorless form occurs with regional differentials, and color returns with readmission or increase of oxygen. The oxygen decrease is readily brought about by the oxygen uptake of the animals. With non-motile material, e.g., eggs or