[1] Hughes, R. D., *Genetics*, **24**, 811 (1939).
[2] Spencer, W. P., *Ibid.*, **23**, 169 (1938).

LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS). III

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A new mutant character of rats, "hereditary acholuric jaundice" was described in 1938 by C. H. Gunn of the University of Toronto. It was shown that the anomaly results from an overproduction of bile pigment in consequence of an excessive fragility of the erythrocytes. The affected individuals are recognizable by their yellow color at or soon after birth. The yellow pigment also enters the developing hair so that white hair of albino and of piebald individuals is distinctly yellowish in color. Growth is usually retarded in affected individuals and in extreme cases "nervous symptoms are developed such as a wobbly gait or partial paralysis, confined chiefly to the hind limbs." Experimental evidence, according to Gunn, indicates that the lag in growth and the nervous symptoms are associated with an inability of jaundiced rats to use carotene as a source of vitamin A, and that they consequently suffer from a prolonged vitamin A deficiency. He shows that the syndrome is inherited as a simple recessive character, which finding is fully conformed by our own observations.

Through the kindness of Professor John W. MacArthur, in whose laboratory the studies of Gunn were made in part, we received in Berkeley a stock of albino jaundiced rats in September, 1938, planning to make a complete study of the linkage relations of the jaundice gene. Dr. MacArthur had already informed us by letter that he had canvassed the question of possible linkage with the commoner mutant rat genes, agouti, albino and hooded, and found no indication of linkage, as recombination occurred freely after crosses involving each of these genes. This finding also we can confirm. The symbol used by MacArthur for jaundiced is *j*, which usage we shall follow.

The mutant genes for which tests have been made by us for linkage with jaundiced are as follows: (1) *A*, agouti; (2) *c*, albino; (3) *Cu*, curly; (4) Cu_2, curly$_2$; (5) *d*, dilute; (6), *h*, hooded; (7) *hr*, hairless; (8) *k*, kinky; (9) *wo*, wobbly. Other mutant genes for which no tests have been made because they are known to be linked with one or another of the genes already enumerated are the following: (10) *b*, brown and (11) *an*, anemia,

which are linked with *Cu;* (12) *l*, Grüneberg lethal, (13) *p*, pink-eye, (14) *r*, red-eye and (15) *w*, waltzing, all of which are linked with *c*, albino.

It may be stated at once that all linkage tests made by us have given negative results, so that for the present jaundiced must be regarded as the marker of an independent tenth chromosome.

A summary statement of the results of the linkage tests is contained in tables 1 and 2.

Tests were made by a backcross to the double recessive in the case of agouti, albino, curly, curly$_2$, and hooded, the expectation being equality of crossover and non-crossover classes, if no linkage exists. Such equality was found within limits scarcely in any case exceeding the probable error, and so without statistical significance. (See table 1.)

F_2 populations (table 2) were considered conclusive evidence of the non-existence of linkage in the cases of dilution, hairless and kinky, in all of which the critical double recessive recombination class, which could arise only from the union of crossover gametes, was found to be close to or in excess of expectation, whereas if linkage existed this class should be below expectation on the basis of free assortment. Also other classes in these F_2 populations were sufficiently close to expectation to negative the idea of linkage.

In the case of wobbly (table 1) an F_2 test was rendered unreliable and a back-cross test between F_1 and the double recessive was rendered impossible by early death of double recessives, none having attained maturity. Accordingly resort was had to the more laborious but more certain method outlined in a previous paper (Castle, 1939). A cross was made between jaundiced and wobbly. F_1 was crossed to animals carrying neither mutant gene. The resulting young were tested individually for presence of one or the other or both of the mutant genes. These tests were made by mating each individual to be tested to an F_1 animal, which from its pedigree would be known to be a carrier of both mutant genes. If there was no linkage between the two mutant genes, those genes would be expected to recombine freely among the gametes produced by an F_1 individual. Such gametes would then be of four sorts equally numerous, *viz.*, (1) *j* only, (2) *wo* only, (3) both *j* and *wo* and (4) neither *j* nor *wo*. No test mating was rated as conclusive unless it resulted in the production of six or more young. Satisfactory tests were made of 143 animals which fall into four classes as follows:

CARRIERS OF *j* ONLY	CARRIERS OF *wo* ONLY	CARRIERS OF BOTH *j* AND *wo*	CARRIERS OF NEITHER *j* NOR *wo*
32	40	23	48

As regards the contribution of the F_1 parent to the animals tested, carriers of *j* only or of *wo* only would arise from repulsion (non-crossover) gametes of the F_1 parent, whereas carriers of *both* or of *neither* would arise from crossover gametes of the F_1 parent. We thus have information as to the nature

of 173 F_1 gametes. These total 32 + 40 = 72 non-crossover gametes, and 23 + 48 = 71 crossover gametes. The two groups are as nearly equal as possible in an odd number of individuals. They furnish a perfect example of free assortment and show conclusively that no linkage exists between *j* and *wo*.

Conclusion.—The recessive mutant gene jaundiced (*j*) shows linkage with no other known rat gene and thus becomes the marker of a tenth independent chromosome pair.

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TABLE 1

SUMMARY OF TESTS FOR LINKAGE BETWEEN JAUNDICED (*j*) AND OTHER MUTANT GENES OF THE RAT

BACKCROSSES BETWEEN F_1 AND DOUBLE RECESSIVES	CROSSOVER GAMETES	NON-CROSSOVER GAMETES	DEVIATION AND *P.E.*
j × agouti	87	81	3 ± 4.4
j × albino	59	59	0
j × curly	257	267	5 ± 7.7
j × curly$_2$	72	84	6 ± 4.2
j × hooded	102	113	5.5 ± 4.9
	Test by "lethal method," Castle, 1939		
j × wobbly	71	72	0.5 ± 4.0

TABLE 2

F_2 POPULATIONS. EXPECTED 9:3:3:1, IF NO LINKAGE EXISTS

j × dilution	*JD*	*jd*	*Jd*	*jd*
	89	29	22	17
Expected	*88.2*	*29.4*	*29.4*	*9.8*
j × hairless	*JHr*	*jHr*	*Jhr*	*jhr*
	95	32	22	9
Expected	*89.0*	*29.7*	*29.7*	*9.9*
j × kinky	*JK*	*jK*	*Jk*	*jk*
	352	108	134	51
Expected	*362.7*	*120.9*	*120.9*	*40.3*

*This is the third in a series of reports on coöperative investigations of linkage in the rat. In the first two papers all observations reported were made by King at the Wistar Institute. In this paper the observations were made by Castle in Berkeley.

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