VARIABILITY OF INCIDENCE AND CLINICAL MANIFESTATION OF MOUSE HEREDITARY MUSCULAR DYSTROPHY ON HETEROGENEOUS GENETIC BACKGROUNDS¹

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AN hereditary muscular dystrophy (symbol *dy*) in the mouse (MICHELSON, RUSSELL and HARMAN 1955) appeared as a deviant in the strain 129/Re, which is highly inbred. All available evidence on the pattern of incidence of this abnormality in 129/Re pedigrees, including the descendants of 129/Re-*dydy* ovaries transplanted to normal host females (STEVENS, RUSSELL and SOUTHARD 1957), supports the hypothesis of an autosomal recessive factor. These data depend, however, upon the expression of the entity against the uniform 129/Re genetic background. No evidence has been published as yet demonstrating expression of the mouse dystrophy syndrome against varying genetic backgrounds. The hereditary muscular dystrophies of man are probably likewise caused by single factor mutations (WALTON 1955), but they occur on backgrounds of extreme genetic variation.

An investigation of the penetrance and expression of mouse muscular dystrophy on various heterogeneous genetic backgrounds was therefore undertaken. The investigation served a dual purpose: (1) to explore the range of variability of the mouse dystrophy syndrome, especially to determine if it would break up into various clinical entities under various conditions of genetic background; (2) to corroborate previous reports that mouse muscular dystrophy is caused by a single recessive mutation. Moreover, the segregating F₂ populations that gave rise to hybrid dystrophics provided the data for linkage tests.

TERMINOLOGY AND METHODS

In the present communication, the term "hybrid" is used with reference to heterogeneous F₂ animals resulting from the cross of two different parent strains or stocks. In a few instances, a more specific use is indicated by qualifying "uniform F₁ hybrid."

We propose to substitute the term *congenic* for *coisogenic* when referring to stocks produced by the introduction of a mutant into an inbred background by

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repeated backcrosses. In this connection the latter term has been used with qualifications by various authors, since the Greek prefix *iso* seems to imply perfect identity of genomes which may rarely or never be real. The Latin prefix *con*, on the other hand, denotes variable degrees of joint qualities without an implication of absolute identity. With reference to stocks produced by the occurrence of a single mutation within an inbred strain, on the other hand, where the new mutation is maintained by forced heterozygosis or by continued backcrossing, we consider the use of the term coisogenic justified and preferable to congenic.

The animals used in this analysis were derived from inbred strains, congenic stocks backcrossed to C57BL/6, and mutant stocks, all maintained at the Jackson Laboratory. Strain designations in the present report are in accordance with SNELL *et al.* (1960). Complete strain symbols are given in Tables 1 and 2; throughout the text, somewhat abbreviated forms are predominant. Mutant symbols, in accordance with GRÜNEBERG (1952), are as follows:

- Linkage group I—chinchilla (c^{ch}), albino (c), and pink-eye (p)
 II—dilution (d)
 V—light-bellied agouti (A^w), black-and-tan (a^t), non-agouti (a), Danforth Short-tail (Sd)
 VI—Caracul (Ca), Naked (N)
 VII—Alopecia (Al), *Rex* (Re)
 VIII—brown (b)
 IX—Brachy (T)
 XI—Microphthalmia-White (Mi^{wh})

TABLE 1

Inbred strains, congenic stocks and mutant stocks used in raising hybrid F_2 populations from outcrosses with 129/Re-dy or C57BL/6-dy

Strain or stock	Genotype	F_1 mated to
*129/Re-dy	$A^wA^wc^{ch}c^{ch}ppdydy$	
†C57BL/6-dy/Re	$aadydy$	
*BALB/c J	$bbcc$	litter mate
*C3H/He J	+	litter mate
*C57BL/6 J	aa	litter mate
*DBA/1 J	$aabbdd$	litter mate
*DBA/2 J	$aabbdd$	litter mate
†C57BL/6- Mi^{wh} /Re	$aaMi^{wh}+$	C57BL/6-Dydy
MI	$a^t t^t Mi^{wh}+$	C57BL/6-Dydy
*AL/Ln	$aaAlAl$	C57BL/6-Dydy
CA	$aaCaCaSp+$	129/Re-Dydy
EI	$Re+Sd+Va+$	129/Re-Dydy
IC	$icic$ (segregate a, b)	litter mate
JE	$aafruie/ruje$	litter mate
YX	$a^t a^t Mi^{wh}+N+T+$	C57BL/6-Dydy

* Inbred strains.

† Congenic stocks.

- XII—ruby (*ru*), jerker (*je*)
 XIII—Spotch (*Sp*)
 XIV—flexed-tail (*f*)
 XVI—Varitint-waddler (*Va*)
 unlinked—ichthyosis (*ic*) and dystrophic (*dy*).

Host females bearing 129/Re-*dydy* ovaries (STEVENS *et al.* 1957), or, in the matings with stocks CA, JE and YX, C57BL/6-*dydy* ovaries, were mated to males of the strains and stocks listed in Table 1. Heterozygous F₁ animals were mated to litter mates in linkage tests of *dy* with recessive marker factors, and with either 129/Re-*Dydy* or C57BL/6-*Dydy* dystrophy carriers in tests with dominant markers. The matings of 129/Re-*dy* with BALB/c, C3H/He, C57BL/6, DBA/1 and DBA/2, respectively, were set up with the major goal of collecting 200 dystrophic hybrids from each group for a comparative longevity study. The primary object of the remaining combinations was to produce additional hybrid genetic backgrounds for the study of dystrophy expression and to provide data for the investigation of genetic linkage between *dy* and the various marker loci.

The reason for introducing *dy* into linkage test matings on two different genetic backgrounds, 129/Re or C57BL/6, consisted primarily in the need to prevent unfavorable pigment phenotypes from appearing in the segregating F₂. C57BL/6-*dy* animals were derived from a project of transferring *dy* onto a second highly inbred genetic background, in addition to 129/Re on which it had occurred. The transfer was carried out by continued back-crossing of host females bearing *dydy* ovaries to males of the C57BL/6 strain. The dystrophy carriers of the F₁ generation (C57BL/6-*Dydy*) were mated *inter se*, and black

TABLE 2

Ratio of dystrophics (in percentage) segregating among F₂ hybrid populations from outcrosses of 129/Re-dy or C57BL/6-dy with several strains and mutant stocks

Parent strain or stock used for <i>dy</i> -hybrid	Percent dystrophic	n†	Collection period‡
BALB/c J	22.3	1192	5/57– 5/59
C3H/He J	14.6	1796	1/57–11/59
*C57BL/6 J	23.2	1628	1/57– 8/58
*DBA/1 J	21.4	1158	5/57– 5/60
*DBA/2 J	20.4	1250	7/57– 9/58
*C57BL/6- <i>Mi^{wh}</i> /Re	23.2	198	1/59–12/59
*MI	26.4	326	10/58–12/59
*AL/Ln	20.6	257	1/59– 6/60
*CA	22.4	125	12/59– 7/60
*EI	22.9	414	1/60– 6/60
*IC	18.9	222	11/58– 9/59
*JE	24.0	438	11/59– 8/60
*YX	16.1	274	11/59– 6/60

* Populations used for linkage tests.

† Total number of F₂ classified.

‡ Month and year of first and last litter born.

dystrophic females of the F_2 generation served as ovary donors for the new backcross generation. The host females were obtained from mating C57BL/6- a^t with 129/J mice. In the resulting uniform F_1 hybrid, a^t and A^w served as dominant marker factors for possible offspring from regenerated host ovarian tissue.

Diagnosis of inbred as well as of hybrid dystrophic animals was based largely on the symptoms by MICHELSON *et al.* (1955). If animals of weaning age, i.e., about four weeks old, are held up by the tail, dystrophics are usually spotted at once by a characteristic clutching or crossing of the hind legs. The prevalent weakness of the hind legs is easily recognized by the lack of physical resistance when the legs are passively stretched out behind the animal, by the tendency of an animal to drag the stretched hind legs for a period rather than to walk on them immediately, and by the rapid exhaustion of dystrophics hanging from the rim of a glass jar by the hind legs. Occasionally a dystrophic animal displays a peculiar, apparently convulsive upward nodding of the head which jerks to an angle of 90° or over from the normal position. The upward jerking is accompanied by gasping. Animals exhibiting one or more of the symptoms described were diagnosed as dystrophic. Young litters were submitted to the same tests beginning at two weeks after birth, in order to determine the time and sequence of onset of clinical symptoms.

Segregating F_2 litters were classified four to five weeks after birth. In a few cases, positive diagnosis for muscular dystrophy had to be postponed for one or two additional weeks. Young animals that died before weaning date were included in the segregation records if classification for each of the loci being tested for linkage was accomplished before death. All dystrophic animals (*dydy*) in the hybrid F_2 populations from matings of 129/Re-*dy* with BALB/c, C3H/He, C57BL/6, DBA/1 and DBA/2 were saved for determination of lifespan.

RESULTS

For the convenience of discussion, the results are arranged into three groups and presented as (1) dystrophy ratio, (2) clinical expression and (3) linkage studies. The lifespan data, which are incomplete at the time of reporting, will be discussed in full in a separate communication.

Dystrophy ratio: Hybrid F_2 populations segregating for the *dy* factor have been produced from 13 different types of parent combinations (Table 1). Dystrophic phenotypes have appeared in all these populations. Moreover, they have in all cases segregated in ratios approximating the theoretical expectation of 25 percent for inheritance by a single recessive factor. The data are summarized in Table 2. The small but persistent deficiency of dystrophics is presumably due either to selective preweaning mortality of dystrophic individuals or to incomplete penetrance of *dydy*.

The most critical evidence to demonstrate that the dystrophy factor is an hereditary entity was obtained from the continued backcrosses of *dy* into the inbred C57BL/6 genetic background. Through the six backcrossing cycles that are completed at the time of reporting, only the third backcross, with 17.6 percent, has

a dystrophy ratio significantly different from the expected 25 percent (Table 3). This low ratio appears erratic, however, and may be attributed to selective mortality of dystrophics or to some other nongenetic interference. The brief period during which offspring from each backcross generation were accumulated, e.g. November 1958 to April 1959 for the group in question, suggests the possibility of erratic results due to seasonal exogenous factors. The persistence of nearly 25 percent incidence, in spite of a change of approximately 98 percent of the original 129/Re genotype to that of C57BL/6, strongly supports the interpretation that muscular dystrophy in the mouse is transmitted as a single factor.

It is probable, however, that the aberrant dystrophy ratio of only 14.6 percent in the large F_2 hybrid group from a 129/Re-*dy* $\times$ C3H/He outcross (Table 2) is caused by genetic modifiers. The dystrophy incidence in the data from this outcross is even lower than from the exceptional third backcross generation of *dy* $\times$ C57BL/6, the sample is much larger, and the degree of significance very high. Moreover, the C3H F_2 hybrids were collected continuously between January 1957 and November 1959, so that seasonal factors, which would tend to be compensated, would not be expected to lead to such a highly erratic result. If excessive preweaning mortality of dystrophics were responsible for their observed deficiency in this F_2 population, the total preweaning mortality would be raised. However, total preweaning loss in the C3H F_2 (25.4 percent) is not significantly greater than in three other F_2 outcross populations (129/Re $\times$ BALB/c 24.4 percent, 129/Re $\times$ DBA/1 23.5 percent and 129/Re $\times$ DBA/2 23.9 percent) which show higher incidence of dystrophy (Table 2). It is much more probable, though not absolutely proven, that the deficiency of dystrophics on the 129/Re $\times$ C3H F_2 population is due to reduced penetrance of the *dydy* phenotype on this particular genetic background.

Additional positive evidence favoring the view of background factors influencing dystrophy penetrance is derived from experiments still in progress with a *dy* $\times$ MI hybrid group (LOOSLI and SOUTHARD, unpublished). Dystrophy symptoms in animals from this cross are almost always distinct at two weeks of age, and their further progress is unusually rapid. In several statistically homogeneous

TABLE 3

Ratio of dystrophics (in percentage) segregating from consecutive backcrosses of dy to inbred C57BL/6

Backcross generation	Percent dystrophics	n*	Collection period†
1	23.2	1628	1/57- 8/58
2	23.4	111	6/58-10/58
3	17.6	170	10/58- 4/59
4	24.1	257	3/59- 8/59
5	25.6	242	7/59- 1/60
6	27.5	40	2/60- 4/60

* Total number of segregating animals classified.

† Month and year of first and last litter born.

groups of hybrids F_2 and F_3 populations, dystrophy segregation is significantly higher than 25 percent and reaches 32 percent ($P = 0.01$) in one group of 266 individuals. It is our working hypothesis that this abnormal segregation, which cannot be explained by postnatal selection, is due to genetic modifiers. The genetic action of this system of modifiers is not yet understood, and there is no indication revealing its physiological mode of action.

Clinical expression: In its major characteristics, the clinical manifestation of *dydy* is very similar on the various backgrounds under investigation. A pronounced weakness of the hind legs is the prevalent symptom in the 129/Re-*dy* and C57BL/6-*dy* as well as in all the hybrid dystrophics. The first indication of weakness frequently consists of a slightly irregular, waddling gait which may be visible as early as twelve days after birth. The positive identification of dystrophics is based principally on the dragging of the hind legs. It may be induced by forcing the legs into a stretched position as early as two weeks after birth. At four weeks of age, spontaneous dragging may be observed occasionally, and in most of the cases it can be induced by manipulation. (Diagnosis of a very few animals, which later show the dystrophy symptoms, is still questionable at four weeks). At older ages, the weakness of the hind legs progresses further until they are practically immobile and useless. In this final stage, which may be reached at about four to five months, the hind legs are extended backward and bent upward. Meanwhile, the animal retains the forelegs in a usable state up to its maximum age of over two years.

The development of clinical symptoms was studied by routinely checking young litters from the five largest hybrid F_2 populations (Table 2) at weekly intervals up to weaning age. Variations between hybrid groups were thereby noted by subjective observation. The progressive sequence of dystrophy symptoms follows the same general pattern in all five groups. Except for the C3H hybrids, the mean age at which dystrophy could be positively diagnosed in a sample of 50 dystrophic animals lay between 19 and 20½ days after birth, with a range from 12 to 35 days. In a sample of 70 dystrophic C3H hybrids, however, the mean age of diagnosis was 28½ days, with a range from 15 to 93 days. On 19 animals from this group, final diagnosis could not be made until more than 30 days after birth, and in four among these 19 animals there was no indication of dystrophy up to 30 days.

By subjective standards, the clinical expression of dystrophy in C3H hybrids was less severe than in other hybrids. A spontaneous display of typical symptoms was rare up to weaning age. Usually the animals had to be overexercised before the muscular weakness was noticeable. Among the remaining four groups, clinical expression appeared slightly more pronounced in the C57BL/6 hybrids than in the other three. At weaning age, they were usually diagnosed without manipulation.

A qualitative difference of a dystrophy symptom between hybrid *dydy* and 129/Re-*dydy* was found in the pattern of convulsive head-jerking. Where in the 129/Re-*dy* the pattern is always the head-jerking and gasping described above, in hybrids it is frequently modified to jittery head motions, with the front

paws "rubbing the nose" as if to hold the head from shaking. The typical jerking has also been observed occasionally in hybrids, however, so that it cannot be considered highly specific for 129/Re-*dy*.

Before weaning, 129/Re-*dydy* animals frequently suffer from an eye infection which appears to be conjunctivitis. The infection responds poorly to oral terramycin treatment, but local application of bacitracin appears to be beneficial. Without treatment the infection usually becomes chronic or may lead to an abscess destroying the eye. This infection is rarely found in hybrids, probably because they are more resistant due to "hybrid vigor."

Linkage tests: The segregation in F_2 of the mutant loci listed in Table 1 was used to test for possible genetic linkage with the *dy* locus. The linkage observations involved a total of 16 marker loci representing 11 linkage groups (GREEN and DICKIE 1959), and the independent locus *ic*. A^w , *p* and c^{ch} were tested with *dy* in coupling, all other markers with *dy* in repulsion. Only Splotch (*Sp*) could not be used for testing because its heterozygous expression was lost in the F_2 .

No convincing evidence of linkage resulted from this series of linkage tests. The possibility of loose linkage to the *p* locus (pink-eye, linkage group I) or to the *Sd* locus (Danforth's short tail, linkage group V) could not be ruled out on the basis of present data, however, and needs further testing. The remaining 15 loci have given no evidence of linkage with *dy*.

DISCUSSION

Dystrophic mice have been obtained in frequencies approaching theoretical expectation among the hybrid F_2 generations of all the 13 strains and stocks of mice into which the original 129/Re-*dy* has been crossed. Moreover, upon continued backcrossing of *dy* onto the inbred C57BL/6 genetic background, the ratio of dystrophics has satisfied theoretical expectation despite the very high degree of "dilution" of the original 129/Re background. The conclusion that hereditary muscular dystrophy in the mouse is caused by a factor behaving as a single, recessive mutation is thereby corroborated.

In all the hybrids so far observed, the clinical expressions of the dystrophy are essentially uniform. The pronounced weakness of the hind legs, contrasted to a remarkable fitness of the front legs, was observed without any exception. The variability of the syndrome was confined to quantitative characteristics, such as time of clinical onset, and the ranges of variability in different groups overlapped each other widely. The dystrophy syndromes in different hybrids clearly present one clinical entity, corresponding to one underlying hereditary entity.

This situation parallels the cases of human hereditary dystrophies where three fairly distinct clinical entities, described as Duchenne type (sex-linked recessive), limb-girdle type (autosomal recessive) and facio-scapulo-humeral type (autosomal dominant), appear to be caused by different factors (BECKER 1957, 1958; CHUNG and MORTON 1959). That there are at least three hereditary factors is indicated by three different modes of inheritance. The possibility that

various distinct mutations may cause only one clinical pattern still exists. But it is clear that each dystrophy mutation affects muscles selectively in a characteristic pattern of regional specificity, which persists through consecutive generations of each pedigree and is not affected by altered genetic backgrounds. With the large number of hybrid mouse populations now investigated, the same constancy of regional specificity can be stated for the action spectrum of the *dy* factor in the mouse.

Some discrepancy between mouse muscular dystrophy and each of the three major human dystrophy types is consistent throughout the hybrid populations. In all groups of mice so far observed, dystrophy resembles the human Duchenne type in its clinical aspects, including differential involvement of the hind legs. However, unlike the Duchenne type, it is transmitted as an autosomal recessive. Although mouse muscular dystrophy and limb-girdle type dystrophy in man are both caused by autosomal recessive factors, the clinical expressions of the two disorders are markedly different. In the mouse, severe posterior limb impairment always precedes the mild anterior limb weakness. In limb-girdle dystrophy, on the other hand, the upper limbs may become involved first, and in advanced stages they are severely affected. The question whether the disorders in the mouse and in humans are caused by analogous factors affecting the same target area in a similar way, or if the mode of transmission of homologous loci has become different in the rodent and in man, cannot be resolved at present. In utilizing the dystrophic mouse as a model in clinical research, it should therefore be remembered that it is still unknown how closely the model actually fits any of the human disorders.

In the cases of abnormal dystrophy ratios presented above, it was suggested that genetic modifiers were responsible for significant deviations from expectation. Among the various mechanisms that are known to cause abnormal segregation, and that may be attributed to controlling hereditary factors, a modified degree of penetrance of *Dy* versus *dy* appears especially feasible. In this hypothesis, high incidence would be caused by the occurrence of phenotypically dystrophic, heterozygous *Dydy* animals, while incomplete penetrance of *dy* even in homozygous doses would lead to low incidence. We observed lessened expression of the syndrome among dystrophics of the low-incidence *dy* × C3H hybrids, and on the other hand very pronounced expression in the high-incidence *dy* × MI hybrid group. This observation lends support to the concept of modified penetrance, even though penetrance and expressivity are not invariably correlated.

It is interesting in this connection to remember that investigations of sporadic cases of human limb-girdle dystrophy have led to the same assumption of occasional expression of dystrophy in the heterozygous state (MORTON and CHUNG 1959). CHUNG, MORTON and PETERS (1960) reported on the case of a carrier mother of two Duchenne boys who developed typical limb-girdle dystrophy symptoms. They interpret this as a case of dystrophy expression in the heterozygous state. Among the mice of various uniform F_1 hybrid genetic background types which from their ancestry were known *Dydy* heterozygotes, however, no animal has yet developed the characteristic dystrophy syndrome. The expres-

sion of dystrophy in heterozygous carriers, a phenomenon presumably discovered in humans, therefore still needs to be demonstrated by direct evidence in the mouse.

SUMMARY

Thirteen different populations of heterogeneous hybrid dystrophic mice were produced by crossing the *dy* mutation from the inbred 129/Re strain, where it had originally occurred, into other stocks and strains of mice. All results clearly supported previous reports that mouse muscular dystrophy is transmitted by a single autosomal recessive factor. Despite the heterogeneity of the dystrophics obtained, the clinical symptoms of the disease were not markedly different from their expression in inbred animals. It is concluded that the characteristic pattern of mouse dystrophy, in analogy to each of several distinct clinical entities known in man, is subject to effects of genetic modifiers only to a minor degree. Results from a continued backcross of *dy* to the C57BL/6 background further confirm this conclusion. Evidence was found, however, suggesting genetic modification of the penetrance of mouse muscular dystrophy. No definitive evidence of genetic linkage of *dy* to any of the mutations used in the experiment was found. Comparative aspects of hereditary muscular dystrophies in man and in the mouse are discussed.

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