Evidence on Inheritance of Muscular Dystrophy in an Inbred Strain of Mice Using Ovarian Transplantation.* (23153)

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(Introduced by C. C. Little)
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An inherited muscular dystrophy which occurs in inbred strain 129 mice has been reported by Michelson, Russell, and Harman (1). The disease was described as a primary myopathy characterized by ataxia, atrophy, and paralysis, and is similar to the Duchenne type of human muscular dystrophy. Evidence was presented which suggested that the abnormality occurs in animals homozygous for a recessive autosomal mutation to be called *dystrophia muscularis* and designated by the symbol *dy*. Muscular dystrophies with a unit hereditary basis are known in other species. There are 3 commonly occurring types of this disease in humans, all hereditary(2,3,4,5). The Duchenne type is evidently transmitted by a sex-linked recessive gene. The facioscapulohumeral type appears to be inherited by an autosomal dominant factor, although the possibility that sometimes it may be sex-limited (to females) has not been ruled out. Limb-girdle muscular dystrophy is probably inherited as an autosomal recessive character, although in some pedigrees the gene appears to have a dominant effect. A muscular abnormality in New Hampshire fowl which grossly and microscopically resembles muscular dystrophies in man has been reported by Asmundson and Julian(6). Judging from data derived from various types of matings, the abnormal birds are homozygous for an autosomal recessive gene (*am*). The potentialities of hereditary muscular dystrophy in mice as a research tool in the study of muscular disease made it desirable to consider procedures which would facilitate production of affected animals in quantity. Russell and Douglas(7), using the technic of ovarian transplantation developed by Robertson(8), demonstrated that offspring can be obtained from transplanted embryonic ovaries, and suggested that this technic could be a valuable tool in experimental zoology. In particular they mentioned that ovaries of lethal genotypes could, when grafted to normal hosts, make possible a direct genetic test of the lethal type itself. This method has since been successfully used to obtain large numbers of offspring by Russell and Hurst(9), Little *et al.*(10), and Stevens(11). The less economical procedures of artificial insemination and ova transplantation were considered impractical for use in obtaining large numbers of offspring. Russell and Hurst(9) worked out a method of using "stock 129" (now called strain 129) mice as donors of ovarian grafts, and as hosts F_1 hybrids with strain 129. This system exploits the fact that strain 129 mice are maintained with forced heterozygosity for a coat color gene, and has the distinct advantage of enabling the investigator to identify each offspring positively as derived from the graft or the reconstituted host ovary. Although the breeding data presented for mouse dystrophy by Michelson *et al.*(1) strongly suggested unit autosomal recessive transmission, 2 characteristics of the entity make it difficult to obtain evidence directly supporting this hypothesis. One is the impossibility of recognizing carriers phenotypically, which led to the incorporation into the pedigree of many matings with at least one non-carrier parent. The second difficulty is the short life-span and physical disability of the dystrophic mice, which prevents successful mating. The evidence presented by these authors was the incidence (25/117, or 21%) in F_2 descendants from ovaries of 2 dystrophic females grafted homoiotopically into normal hosts.

In this paper we present further evidence based on matings of animals of identified genotype which confirms the hypothesis that

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dystrophia muscularis is inherited as a unit recessive. Furthermore, an example is given which illustrates that the technic of ovarian transplantation is practical in obtaining large numbers of offspring from ovaries grafted from females of extremely low fecundity.

Materials and methods. Strain 129 mice have been inbred for more than 20 generations by mating brothers and sisters heterozygous for a pair of alleles which determine whether the animals are albinos or pigmented. The colors resulting from recombinations of these alleles are: white ($c^a c^a$), chinchilla ($c^{ch} c^{ch}$), and a hue intermediate between these ($c^a c^{ch}$). In most cases, chinchilla females with muscular dystrophy (*dy dy*) were used as donors of ovarian grafts. The hosts were black agouti females obtained from mating albino strain 129 with DBA mice. The technic of ovarian transplantation is essentially that described by Robertson(8), although a few modifications have been introduced. Each operation was performed with clean instruments, including Swiss-made watchmaker's forceps with fine points, iridectomy and dissecting scissors. The animals were anaesthetized with ether. The donor was sacrificed and ovaries removed from the ovarian capsules, bisected, and kept moist within the body cavity of the donor until the host was prepared to receive the graft. A mid-dorsal incision approximately 1 cm in length was made through the skin at the level of the ovaries. The incision was drawn laterad and a second incision, through the body wall, was made just ventral to the dorsal muscle mass, and the ovary was exteriorized by pulling out the fat pad attached to the ovarian capsule. A small cut was made in the ovarian capsule so it could be peeled back to expose the ovary. The ovary was removed by pinching it off at the hilus, and the graft of ½ an ovary was inserted into the empty capsule. It is important to remove as much of the ovary as possible so that reconstitution will not occur. The encapsulated graft was then inserted into the body cavity. The skin incision was drawn to the other side of the animal and the remaining host ovary with its capsule and oviduct was removed. The skin incision was closed by a wound clip. It has been established that when donors approximately 6 weeks old are used, grafts of one half of an ovary perform, reproductively, better than grafts of a whole ovary or ¼ of an ovary (Stevens, unpublished). The hybrid females, bearing grafts from dystrophic donors were mated with normal (*Dy Dy*) or heterozygous (*Dy dy*) males. The normal males were obtained from a colony of strain 129 mice known to be free from dystrophic individuals. Two classes of offspring, unaffected (*Dy dy*) and dystrophic (*dy dy*) resulted. The normal heterozygous offspring were mated *inter se*. All heterozygous animals used in breeding experiments were offspring derived from *dy dy* ovarian grafts.

Results. Reproductive performance of animals bearing ovarian grafts. The reproductive performance to date (2/7/57) of animals bearing ovarian grafts from donors with muscular dystrophy is summarized in Table I.

Since the grafts consisted of halves of ovaries, approximately 75 dystrophic donors were used. Of 294 females with ovarian grafts, 205 (69%) have produced 2348 graft offspring which have survived until weaning age. Only 39 of these females which have produced exclusively graft offspring have been observed throughout their reproductive period, the remaining ones being currently under observation. The average number of litters from the 39 mice was 5.1 per female and the average litter size at birth was 4.1.

TABLE I. Reproductive Performance of Normal Females Carrying Ovarian Grafts from Donors with Muscular Dystrophy.

	No. females producing offspring from:				No. offspring weaned:*	
No. operations	Graft ovary	Host ovary	Mixed	No. offspring born	Graft origin	Host origin
294	150	4	55	3234	2348	162

* Some mice younger than weaning-age were used for experimental purposes.

TABLE II. Classification of Offspring Derived from Matings of *Dy Dy* Females Carrying *dy dy* Ovarian Grafts and from Matings of Heterozygous Animals (*Dy dy*) *inter se*.*

Parent genotype	No. weaned	No. weaned with muscular dystrophy	%
dy dy ovarian graft × *Dy Dy* ♂	815	0	0
dy dy ovarian graft × *Dy dy* ♂	1413	626	44
Dy dy ♀ × *Dy dy* ♂	2211	422	19

* Individuals from host or mixed litters or litters from which animals younger than weaning age were removed for experimental purposes are not included in this table.

The number of litters per female ranged from 1 to 9 and the size of the litters varied from 1 to 11. These data make it apparent that the technic of ovarian transplantation is practical for use in direct genetic studies using gonads from genotypically lethal mice. In addition, the technic is shown to be satisfactory in producing large numbers of offspring.

Genetic segregation of Dy and dy alleles. The results of breeding F_1 hybrid females bearing ovarian grafts of the genotype *dy dy* with normal (*Dy Dy*) and heterozygous (*Dy dy*) males are presented in Table II along with the results of breeding heterozygous animals (*Dy dy*) *inter se*. All *Dy dy* individuals used as parents were offspring of *dy dy* grafted ovaries. Although mice with muscular dystrophy can usually be distinguished from their normal littermates at 2 weeks of age, we have included in our results only animals which survived to weaning age when in all cases the disease can be positively identified.

When females with ovarian grafts from dystrophic donors were mated with *Dy Dy* males, all of the offspring were unaffected. When these females were mated with heterozygous males, slightly less than half (44%) were affected with the disease. *Inter se* matings (*Dy dy* × *Dy dy*) of offspring of transplanted ovaries yielded 19% affected young. Male and female offspring were affected in approximately the same numbers. The proportions of dystrophic animals resulting from these 3 types of matings approach those that would be expected if the affected animals were homozygous for an autosomal recessive gene.

Discussion. An example is provided here of use of the technic of ovarian transplantation to facilitate genetic study of an abnormality resulting in early death and drastically reduced fecundity. Russell and Douglas(7) have suggested the use of this procedure to permit direct breeding tests of lethal genotypes.

To make use of the technic of ovarian transplantation, it is necessary that donors and hosts be histocompatible. Furthermore, the donor-host combination must be such that offspring derived from the graft can be distinguished from those derived from possible reconstituted host ovarian tissue. In some cases, the latter requirement is easily met by use of a genetic marker. It is particularly fortunate that the *dy* mutation occurred in the inbred strain 129 which is maintained heterozygous for a coat color gene.

On the basis of breeding data of heterozygous offspring (*Dy dy*) of transplanted ovaries, Michelson *et al.*(1) suggested that *dystrophia muscularis* is caused by homozygosity for a single mutant autosomal recessive gene. Our larger body of data completely confirms this hypothesis. The proportional deficiency of observed vs. expected dystrophics is approximately the same in matings between heterozygotes (19% instead of 25%) as in matings between heterozygous males and animals with homozygous ovarian grafts (44% instead of 50%). One possible explanation for this deficiency is differential mortality of genetically dystrophic animals before they could be classified phenotypically. An alternative explanation was presented by Michelson *et al.*(1) who suggested that the expression of the gene may be masked occasionally by its environment. It is possible that both differential mortality and incomplete penetrance are involved.

The genotype of offspring of transplanted *dy dy* ovaries can always be accurately assigned, since all must contain at least one *dy* gene. These animals provide a reliable supply of known *Dy dy* heterozygotes for use in production matings. The positive identifica-

tion of parental genotypes with respect to the *Dy* and *dy* alleles provides a sound basis for expecting certain ratios of normal:affected offspring. This is particularly useful in the search for early differences between normal and affected individuals.

The method of ovarian transplantation has the added advantage of putting to use the germ cells of affected homozygotes which would otherwise be unavailable for reproductive purposes. Compare the incidence of dystrophic animals in the 1951-55 breeding colony of strain 129 mice with that in the 1956 colony maintained with the help of ovarian transplantation. Between 1951-55, in spite of considerable effort toward selection of matings between heterozygotes, the incidence of dystrophics was 6% (177/2950)(1). In 1956, the combined incidence of dystrophics among offspring of the 3 types of matings listed in Table II was 1048 4439, or 24%. The use of animals with ovarian grafts has greatly facilitated the production of dystrophic mice in quantity and has permitted direct genetic tests which have provided definitive evidence that *dystrophia muscularis* in strain 129 mice is inherited on a unit autosomal recessive basis.

Summary. 1. Critical evidence is given which supports the hypothesis that *dystrophia muscularis* is inherited in strain 129 mice on a unit autosomal recessive basis. 2. Offspring were obtained from ovaries of dystrophic females transplanted to histocompatible normal hosts. The incidence of dystrophy in a large F_2 population descended from these grafted ovaries is 19%, and in a large backcross population 44%. 3. Approximately 70% of the animals bearing ovarian grafts yielded graft offspring. The technic of ovarian transplantation, as modified from that of Robertson is described. 4. Using offspring of known genotype derived from transplanted ovaries, it has been possible to provide a reliable and extensive supply of dystrophic animals and normal littermates for research use.

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Fe^{59} Assessment of Erythropoiesis in Mice Following Injections of Anemic Rabbit Plasma Extracts.* (23154)

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Carnot and DeFlandre(1) postulated the existence of an erythropoietic stimulating factor in plasma of rabbits. Since then much evidence(2-5) has been published favoring a humoral control of red cell production. However, the effectiveness of an erythropoietic factor in deproteinized plasma extract from rabbits made anemic with phenylhydrazine in producing erythropoietic stimulation as judged by Fe^{59} uptake in red cells has not been

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