sera obtained after colchicine treatment. Since the present experiment showed that administration of a high dose of colchicine suppresses antibody response, the reduction in antibody response reported by Forman *et al.* may have been due to the action of the large amount of colchicine accumulated over the 11- to 14-day period. Fagraeus and Gormsen (11) injected 0.5 mg of colchicine/kg body wt. into rats 3 days after immunization and noted that agglutinin response was significantly reduced. Since this is a toxic dose for the rat(12), the reduction in antibody response may, here too, have been the result of the high dosage of colchicine.

Summary. The adjuvant effect of colchicine was investigated in the hamster primarily because this species is unique in tolerating high dosage of colchicine. Administration of non-toxic doses of colchicine enhanced hemagglutinin response, but the increase in antibody response was no greater than that reported for the rabbit, a species far more susceptible to the toxicity of colchicine. The increased level of hemagglutinin was for complete antibody; levels of incomplete antibody were not elevated. Increase in the dose of colchicine to near-lethal levels resulted in considerable reduction in antibody response.

We wish to thank Miss A. Spraggins for technical assistance.

1. Tanaka, N., Coons, A. H., *J. Histochem. and Cytochem.*, 1954, v2, 460.
2. ———, *Bull. N. Y. Acad. Med.*, 1956, v32, 171.
3. Taliferro, W. H., Jarowslow, B. N., *J. Infect. Dis.*, 1960, v107, 341.
4. Orsini, W. M., Pansky, B., *Science,* 1952, v115, 88.
5. Eigsti, O. J., Dustin, P., *Colchicine,* Iowa State College Press, Ames, 1955, p179.
6. Turbyfill, C. L., Soderwall, A. L., *Science,* 1957, v126, 749.
7. Cardinali, G., Cardinali, G., Handler, A. H., Agrifoglio, M. F., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 891.
8. Jarowslow, B. N., *Radiation Research Suppl.,* 1961, in press.
9. Johnson, A. G., Gaines, S., Landy, M., *J. Exp. Med.,* 1956, v103, 225.
10. Forman, C., Seifter, J., Ehrich, W. E., *J. Allergy,* 1949, v20, 273.
11. Fagraeus, A., Gormsen, H., *Acta path. microbiol. scand.,* 1953, v33, 421.
12. Poe, C. F., Johnson, C. C., *Acta Pharmacol. et Toxicol.,* 1949, v5, 110.

Received December 11, 1961. P.S.E.B.M., 1962, v109.

Production of All-dystrophic Litters of Mice by Artificial Insemination.* (27291)

H. G. WOLFE AND JANICE L. SOUTHARD (Introduced by N. Kaliss)
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Dystrophia muscularis (gene symbol *dy*) is a primary myopathy in mice(1) that occurred as a spontaneous mutation in strain 129/Re, maintained by Dr. E. S. Russell of the Roscoe B. Jackson Memorial Laboratory. It is transmitted as an autosomal recessive, and its expression is remarkably constant on a large number of genetic backgrounds(2). A characteristic feature of the disease is pronounced weakness of the hind legs, and early death.

* This study was supported by a grant to Roscoe B. Jackson Memorial Laboratory from Muscular Dystrophy Assns. of America.

It is highly desirable to have available dystrophic tissue collected at prenatal and early postnatal stages, when clinical diagnosis is not possible. It may then be possible by retrograde histological and biochemical analysis to determine the primary cause of the myopathy. The earliest possible positive identification of dystrophic mice has been approximately 2 weeks after birth. Since dystrophic males rarely breed, it is impractical to use natural mating to obtain 100% dystrophic litters; neither are there any linked genetic characters known which could assist

in early identification of dystrophic animals in segregating litters.

The production of all-dystrophic litters has been achieved by artificial insemination of dystrophic juvenile or young adult females with sperm collected from dystrophic males. This report describes the technics used, and compares reproductive potentials of the different strains and genotypes involved.

Materials and methods. For the most part, inbred 129/Re and 129/Re-*Dydy* × C57BL/6-*Dydy* F_1 hybrids (hereafter referred to as 129B6F_1 hybrid) were used. The genetic attributes and a comparison of syndrome characteristics in *dydy* mice from these 2 genetic sources are given in(3). The F_1 hybrid dystrophic has increased vigor and longevity (but still exhibits a typical clinical picture) and, therefore, seemed to offer the best chances for success. Some miscellaneous F_2 generation mice from linkage tests are also included in the data.

Different methods of artificial insemination were tried at the outset. Only the most successful of these are reported here, namely insemination *via* laparotomy of young adult or immature females in whom ovulation had been hormonally induced. Insemination of primed females was done in groups of about 20 mice and the experiment was designed so that some females in each group were allowed to copulate with normal males as a measure of optimum breeding performance. Single injections of pregnant mare serum (PMS) of 2.5 I.U. or 5.0 I.U. were usually given, followed by a single ovulating dose of 2.5 I.U. of human chorionic gonadotropin (HCG). Both PMS and HCG were made from stock† to contain 10 I.U. per ml of 0.85% saline and were administered intraperitoneally. The interval between PMS and HCG ranged from 36 to 38 hours; that between HCG and insemination or normal copulation ranged from 12 to 18 hours.

Hormonally treated females were placed with vasectomized males usually at 10 hours post-HCG injection and remained for 2 to 6 hours, or until discovery of a vaginal plug. In some groups all females were inseminated. In other groups, only those females having vaginal plugs were inseminated. There was little risk from duct regeneration in the vasectomized male; without exception a section was removed from each duct and the severed ends were tied. Most of the vasectomized males were from strain C57BL/6J. The remainder were from strains 129/J, 129/Re, or a partially inbred stock having the mutant character Light (B^{lt}).

Sperm were obtained from males by stripping the ductus deferens, although in some instances sperm from the epididymis were combined with those from the duct. Males were killed by cervical dislocation, and were dissected 20-60 minutes later. The diluent for the sperm was either a 0.85% NaCl solution or a 9.5% reconstituted dried skim milk solution(4,5). The preparations were injected by syringe and 27 gauge needle directly into the ovarian end of the uterine horn. The amount of sperm preparation per injection averaged .02 cc with a mean sperm count of 3.33×10^5. Usually only one uterine horn was injected. Preparatory to this, an incision in the skin was made mid-dorsally in the lumbar region and the uterine horn exposed by another small incision through the lateral body wall just ventral to the dorsal muscle mass. The point to be incised can best be visualized by noting the position of the ovarian fat pad. The skin incision was closed by a wound clip. Operation usually was under nembutal and in some cases ether anaesthesia. This same type of operative technic has been used successfully in ovarian transplantations(6).

The gross morphology and motility of sperm from 129/Re dystrophic males appear normal. However, sperm concentration in the ductus deferens is significantly less than in normal littermates. From the few sperm counts made on 129B6F_1 hybrid males, it appears that there is little if any difference between dystrophic and normal during early reproductive life.

Results and discussion. Table I gives results obtained from inseminations. A total of 13 litters was produced with a mean litter size at birth of 3.9. Best results were obtained using 129B6F_1 hybrid females, which gave

† Ayerst Laboratories, Inc., New York City.

TABLE I. Production of Dystrophic Mice by Artificial Insemination of Hormonally Treated Females.

Sperm source	129/Re females						129B6F$_1$ females						Misc. F$_2$ females					
	Normal			Dystrophic			Normal			Dystrophic			Normal			Dystrophic		
	Litters/ Trials	Mean litter size birth	Mean litter size weaning	Litters/ Trials	Mean litter size birth	Mean litter size weaning	Litters/ Trials	Mean litter size birth	Mean litter size weaning	Litters/ Trials	Mean litter size birth	Mean litter size weaning	Litters/ Trials	Mean litter size birth	Mean litter size weaning	Litters/ Trials	Mean litter size birth	Mean litter size weaning
Normal 129/Re	0/4	0	0															
Dystrophic	0/2	0	0	0/3	0	0				0/1	0	0						
Normal 129B6F$_1$	2/12	5.5	3.0				2/5	3.0	3.0	0/5	0	0				0/2	0	0
Dystrophic	1/11	1.0	1.0	0/3	0	0	4/10[a]	5.0	4.2	2/8[b]	4.0	4.0	1/1	1.0	1.0	1/4[c]	4.0	2.0
Misc. F$_2$ dystrophic							0/2	0	0	0/1	0	0				0/3	0	0

a) Litter of one mouse Caesarean derived and fostered.
b) One female died prior to parturition; one litter fostered to normal female.
c) Litter fostered to normal female.

25.0% success compared to 8.1% in inbred 129/Re females, for strains and phenotypes of males combined. All litters of mice produced by artificial insemination were from 129B6F$_1$ hybrid sperm. It is important to note that no litters were from dystrophic males of strain 129/Re, in a total of 8 trials. This might be a result of the small number sampled, but it might also mean that hybrid males are the source of choice for *dydy* sperm. Hybrid dystrophic females are more fertile than dystrophic 129/Re females, since neither pregnancies nor litters were recorded for the latter. It was sometimes necessary to foster-mother young born to dystrophic females.

TABLE II. Normal Matings[a] of Hormonally Treated Females.

Females	Type	Litters/ Trials	% Partu-rient	Mean litter size Birth	Weaning
129/Re	Normal	14/29[b]	48.3	5.4	3.3
	Dystrophic	1/4[c]	25.0	7.0	0
129B6F$_1$	Normal	22/48	45.8	11.8	9.5
	Dystrophic	7/16	43.8	9.0	3.0
	Totals	44/97	45.4	9.2	6.3

a) Normal males were largely 129B6F$_1$ hybrids; some were (129/Re x C3HeB/FeJ) F$_2$ generation mice, and a few were from strain 129/Re.
b) Two females died prior to parturition.
c) One female died before parturition complete. Seven young were born and 6 fetuses were found on autopsy.

Control matings are shown in Table II. Of those females primed and then mated to normal males, 45.4% bore young. This compares with 16.4% successful artificial inseminations, making the sperm transfer technic itself roughly one-third as efficient. There is a notable difference between normal (*Dy*-) and dystrophic (*dydy*) dams of strain 129/Re in capacity to bear litters; this is not true of the 129B6F$_1$ hybrid.

It is pointed out to potential users of all-dystrophic litters that offspring from *dydy* × *dydy* matings involving at least one F$_1$ hybrid or non-inbred parent will show genetic segregation at loci other than the *dy* locus. While this introduces genetic variation it does not greatly affect expression of muscular dystrophy(*2,3*).

Summary. Three all-dystrophic litters of mice from *dydy* matings were produced by artificial insemination of juvenile or young adult dystrophic females with sperm collected from dystrophic males, following induced ovulation in the female. Fertility and litter size of inbred and hybrid dystrophic (*dydy*) mice are compared to inbred and hybrid normal (*Dy-*) mice. It was found that hybrid dystrophic mice were far superior to inbred dystrophic mice and provided a practical means to obtain all-dystrophic litters. The insemination procedure makes it possible to obtain known dystrophic tissue at prenatal and early postnatal stages for histological and biochemical analyses.

1. Michelson, A. M., Russell, E. S., Harman, P. J., *Proc. Nat. Acad. Sci.*, 1955, v41, 1079.
2. Loosli, R., Russell, E. S., Silvers, W. K., Southard, J. L., *Genetics*, 1961, v46, 347.
3. Russell, E. S., Silvers, W. K., Loosli, R., Wolfe, H. G., Southard, J. L., to be published.
4. Dziuk, P. J., Runner, M. N., *J. Reprod. Fertil.*, 1960, v1, 321.
5. Melrose, D. R., *Proc. IIIrd Int. Congr. Anim. Reprod., Cambridge*, 1956, sec. 3, p68.
6. Stevens, L. C., Russell, E. S., Southard, J. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 161.

Received December 11, 1961. P.S.E.B.M., 1962, v109.

Intestinal pH and Absorption and Deposition of Ca^{47} in the Rachitic Chick.* (27292)

R. H. WASSERMAN AND A. N. TAYLOR
Department of Physical Biology, N. Y. State Veterinary College, Cornell University, Ithaca, N. Y.

It has been generally assumed that radiocalcium after intestinal absorption behaves the same as if introduced parenterally and, therefore, the amount of ingested radiocalcium deposited in the skeleton would reflect absorption(1-4). The validity of this assumption is usually tested by comparing the amount of radionuclide deposited after parenteral administration under a given set of conditions; if (as usually observed) no differences are noted, it would then appear that the experimental situation was without effect on the skeleton *per se* and differences in the radionuclide content of skeleton after oral administration would reflect relative differences in intestinal absorption.

Recently, it was reported that lactose injected with Ca^{45} into the ileal lumen of the rat increased the relative deposition of the absorbed Ca^{45}(5); earlier observations by Vaughn and Filer(6) suggested that Ca^{45} absorbed from a solution containing glucose also was deposited to a greater degree in the femur of rats than that absorbed in absence of the carbohydrate. Thus, it became apparent that, for certain comparisons, the amount of Ca^{45} found in bone was not strictly a reflection of the degree of Ca^{45} absorption.

The effect of vit. D on absorption of Ca^{47} from ligated duodenal segments and the deposition of this radionuclide into the tibia was recently determined in the rachitic chick (7); it was found that duodenal absorption of Ca^{47} with or without vit. D-treatment was directly related with the tibia content of radionuclide. In these studies, the dosing solution containing Ca^{47} was injected into the duodenal lumen at a relatively low pH because, it was reasoned, ingesta normally passed into the duodenum from the gizzard would have a pH of about 2.6(8). Upon further experimentation, it was noted that, at a near neutral pH, proportionally more of the absorbed Ca^{47} was deposited in the tibia of the vit. D-treated chick than in that of the rachitic chick. These and related observations are reported here.

Experimental. One-day-old chicks were placed on a complete growing mash for 5 days, then were given a rachitogenic diet (General Biochemicals, Inc.) *ad lib* for 3 to 4 weeks. After this period, the chicks showed severe signs of rickets. Twenty-four hours before experiment, one-half of the group was given 500 I.U. vit. D_3 orally in Wesson oil and the rachitic controls, Wesson oil alone.

* Supported by Nat. Inst. Health Grant.