L-serine-3-^{14}C and thymidine-^{3}H, aminopterin decreases *de novo* synthesis of DNA-thymine-^{14}C to 16%, while incorporation of thymidine-^{3}H into DNA is increased to 140% of control values. These results are interpreted as evidence for increased utilization of preformed thymidine in DNA synthesis in the presence of aminopterin. The possible mechanisms for this increased incorporation of preformed thymidine into DNA in the presence of aminopterin are discussed.

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Developmental Aspects of Glutathione Levels in Dystrophic Mice.* (32523)

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Studies of nutritionally induced and hereditary muscular dystrophy indicate changes in many enzyme and coenzyme levels(1). Greatly elevated glutathione reductase activities have been reported in dystrophic (dy/dy) mice(2,3). High glutathione (GSH) levels have been reported in the skeletal muscle of vitamin E-deficient rabbits(4,5), and in chickens with hereditary muscular dystrophy(6,7). Contrary results have been obtained for GSH levels in vitamin E-deficient rabbits by other workers(8).

In studies of chick skeletal muscle investigators have reported GSH concentrations which varied with age and sex, not only between normal and dystrophic animals but also among the various sub-lines of the dystrophic forms. In vitamin E-deficient rabbits (8) dystrophic individuals exhibit depressed levels for "sulfhydryl groups," which gradually rise to the "normal" value upon continued exposure to the deficient diet. In a study of muscle dialysates from mice of the 129/J strain, sex unspecified(9) it has been shown that SH concentration is higher in normal controls than in dystrophics, while SS concentration is higher in the dystrophic animals. Of special interest is a study of catheptic and other lysosomal enzymes in dystrophic rabbits, chickens and mice, in which the elevation of such enzyme activity in affected animals is always lowest in mice(10,11).

Apparently neither normal nor dystrophic GSH values for mouse muscle have appeared in the literature. The objective, in this preliminary study, was to ascertain whether a developmental pattern for muscle GSH values exists in both normal mice and those with progressive muscular dystrophy from fetal through adult stages.

Methods. Leg and thigh muscles were removed from 17-112-day-old female mice of

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the 129/J strain, after cervical dislocation. Care was exercised to remove as much fat, connective tissue and blood as possible. Controls were mostly heterozygote (Dy/dy) animals with a few homozygous normal (Dy/Dy) individuals all of which are subsequently designated as controls. The dy/dy animals were littermates of the same sex. Male mice of the same description, 43-104 days of age, were also utilized. Dystrophic fetuses were obtained by artificially inseminating(12) pink-eyed dystrophic females with mixed sperm obtained from brown-eyed Dy/Dy males and pink-eyed dy/dy males. Pink-eyed fetuses so otained are homozygous for dystrophy while their brown-eyed littermates are heterozygous. Several experiments were also performed upon whole mouse heart and cerebrum.

Reduced glutathione (GSH) was determined by the alloxan "305" method of Patterson and Lazarow(13). Excised tissues were homogenized in ice cold 5% metaphosphoric acid utilizing a glass homogenizer. Other homogenizers with blades were unsatisfactory. The homogenate was centrifuged at 13,000 rpm (25,150 RCF $\times$ g) for 10 minutes at $-10°$C. Corrections were made for the volume of tissue fluid added. The remainder of the method is as described by the original authors. The alloxan method has been used previously(5,14) and compares favorably with other methods. Tissue water content was determined by dehydration at 105°C for 24 hours, cooling over silica gel in a vacuum dessicator and subsequently weighing the tissue rapidly on a Sartorius balance.

In our early experiments lipids interfered with the procedure, especially in older mice. Centrifugation as indicated resulted in the formation of a lipid crust over the supernatant. It was found that lipids would adhere to a glass rod inserted through the crust and withdrawn. Recentrifugation demonstrated the efficacy of the method.

Results and discussion. In the postnatal age range studied, the water content of various tissues did not vary greatly from average values of 76% for mouse muscles, 75% for whole mouse heart and 77% for whole mouse brain. In the mouse fetus, 17 days after insemination, the water content was found to be 89%.

As a check on the alloxan method, femoral muscle from a young sexually mature male New Zealand rabbit was excised, frozen on solid carbon dioxide and treated as indicated. Duplicate values of 47.1 mg% wet weight GSH were obtained. This compares with high and low values of 1.88 and 46.3 mg% obtained by Ryerson(5) in control rabbits of the same sex and strain, by the same method. In the cerebral hemispheres of normal 129/J strain mice we obtained an average of 66.1 mg% GSH in two 58-day-old females and 67.9 mg% in a 48-day-old male. These compare favorably with average values for

TABLE I. Levels of Reduced Glutathione in Control and Dystrophic Mouse Skeletal Muscle. Values as mg GSH/100 g wet weight of tissue.

		Normal		Dystrophic
Females	All animals	31.5 ± 3.7 (14)	$P < 0.05$	35.5 ± 5.4 (14)
	17 days	35.7 ± 3.6 (4)	N.S.	40.4 ± 6.4 (4)
		$P < 0.01$		$P < 0.05$
	46-112 days	29.8 ± 2.0 (10)	$P < 0.02$	33.6 ± 3.8 (10)
Males	All animals (43-104 days)	36.4 ± 5.8 (8)	N.S.	37.2 ± 3.7 (8)
		vs females $P < 0.05$		*vs* females N.S.

Data are expressed as mean ± standard deviation.
Numbers in parentheses indicate number of animals in the group.
N.S. indicates P of >0.05; not statistically significant.

cerebral hemispheres from white mice (69.31, 68.56 and 66.31 mg%) obtained by a combustion method(15,16).

The data for GSH levels in skeletal muscle of mice are shown in Table I. Significantly greater concentrations of GSH were found in the skeletal muscle of dystrophic females, compared with their normal littermates, when all animals were considered as a group ($P<0.05$). This difference becomes more apparent when only the older (46-112 day) females are considered ($P<0.02$). In this respect our observations agree essentially with the results in male rabbits(4,5) as well as in both sexes of chickens(6). However, our finding that the GSH content of normal and dystrophic skeletal muscle is significantly greater in younger female (17 day) mice than in older ones, does not agree with the developmental pattern report in chickens(6) or in vitamin E deficient rabbits(8).

The analysis of the data for muscle GSH levels in the male mouse indicates that a difference may exist between normal and dystrophic animals but suggests that in the age range investigated the differences between normal and affected pairs is not significant.

Comparison of values between normal males and females indicates that muscle GSH values of males are consistently higher ($P<0.05$). The same kind of sexual difference has been observed in both normal and dystrophic chickens(6).

Comparison of GSH data from rabbits and chickens(4,5,6,7) with those of mice demonstrates that the degree of elevation in dystrophic mice is much lower than that found in rabbits and chickens. The same observation was made with regard to catheptic activity in these same 3 species(10,11).

The GSH levels in mouse heart (Table II) suggest that no significance can be attached to the difference between normals and dystrophics of either sex. The values for male hearts are generally higher than those of female mouse hearts. In addition it may be noted that GSH levels are higher in cardiac muscle of either sex when compared to skeletal muscle. Similar findings have been reported for the rat(17). At present we are unable to offer any interpretation of the sexual differences of GSH levels observed in skeletal and cardiac muscle although endocrine disturbances have been reported in both sexes of mice with progressive muscular dystrophy (18).

Several litters of "marked" fetuses were produced by artificial insemination. In most litters the number of individuals was either too low or contained a disproportionately high number of Dy/dy fetuses; these were used for other studies in progress. One litter of 10 fetuses was delivered by Cesarean section 17 days after insemination; one fetus was utilized to determine water content. Analyses were performed on whole fetuses. The average

TABLE II. Levels of Reduced Glutathione in Control and Dystrophic Mouse Cardiac Muscle. Values as mg GSH/100 g wet weight of tissue.

		Normal		Dystrophic
Females	All animals	44.9 ± 3.7 (10)	N.S.	42.0 ± 7.4 (10)
	17 days	45.3 ± 5.0 (4)	N.S.	40.3 ± 6.4 (4)
	46-68 days	44.7 ± 3.0 (6)	N.S.	43.2 ± 8.4 (6)
		N.S.		N.S.
Males	All animals (43-104 days)	47.0 ± 2.5 (7)	N.S.	47.3 ± 2.6 (7)
		vs females N.S.		*vs* females N.S.

Data are expressed as mean ± standard deviation.
Numbers in parentheses indicate number of animals in the group.
N.S. indicates P of >0.05; not statistically significant.

value obtained for 6 Dy/dy fetuses as 36.00 ± 1.11 mg% GSH, while the average for 3 dy/dy fetuses was 37.42 ± 0.61 mg%. Only one of the Dy/dy fetuses yielded a higher value than any of the dystrophic fetuses.

In a number of papers(4,5,6,17) no role has been established or suggested for the metabolic function of GSH in normal and dystrophic animals. A sharp rise in muscle lysosomal cathepsins, concurrent with autolysis, has been shown in both vitamin E-deficient and hereditary muscular dystrophies (10,11). The latter workers have postulated that a great part of the observed increase in lysosomal cathepsins might be due to an influx of macrophages. In addition there are indications that a large number of enzyme systems(19) including the cathepsins(20) are activated by GSH. The possibility exists that the greatly increased catheptic activity of dystrophic skeletal muscle requires greater amounts of GSH. The observed increase in GSH may also be related to increased glutathione reductase activity(2,3).

Summary. 1. In the skeletal muscle of normal female mice, the reduced glutathione concentration decreases rapidly from the relatively high 17 day levels to the 46-112 day old normal adult female averages. 2. All the GSH muscle levels in dystrophic females are higher than in their normal littermates. 3. Normal male skeletal muscle yields higher GSH levels than normal female skeletal muscle in the same age range. 4. Comparison between normal and dystrophic males indicates no significant difference in GSH levels in skeletal muscle. 5. At present no significance can be assigned to differences in heart GSH levels for either sex. 6. Comparison with data in the literature indicates that differences in GSH levels in skeletal muscle of dystrophic and normal mice are minimal when compared with those reported in dystrophic and normal rabbits and chickens.

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