Tissue Lipids of *Dystrophia muscularis*, a Mouse with Inherited Muscular Dystrophy.* (23755)

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A rise in cholesterol and total lipid content of skeletal muscle is a prominent feature of nutritional muscular dystrophy induced by the feeding of a Vit. E-deficient ration to guinea pigs, rabbits, rats and calves(1-5). In the rabbit this is accompanied by a hypercholesterolemia(1), and in both the rat and the rabbit increases in the cholesterol content of the brain have been reported(2,4). Increases in the cholesterol level of the muscle have been found in Vit. E-deficient chicks(6) and in patients afflicted with muscular dystrophy(7). Hitherto it was not possible to obtain laboratory animals with dystrophy without a concomitant deficiency in Vit. E. Recently *Dystrophia muscularis*, a mutation in strain 129 mice, with inherited muscular dystrophy, became available(8). Since investigations of the nature of the disturbance in cholesterol metabolism of dystrophic muscle might reveal the underlying metabolic disorder in this disease, in this communication are reported the cholesterol and total lipid content of tissues of *Dystrophia muscularis*.

Procedure. Dystrophic mice and their non-dystrophic littermates were sacrificed under nembutal anesthesia. Blood samples were obtained by heart puncture. Pooled blood samples were centrifuged, and the lipids were extracted from the plasma with ethanol:acetone (1:1). Skeletal muscle, liver and brain were analyzed individually; and kidneys, heart and lungs from animals of the same sex were pooled for analysis. Skeletal muscle, heart and lungs were finely ground with acid-washed sand and anhydrous sodium sulfate; and liver, brain and kidneys were ground with anhydrous sodium sulfate alone. The resulting dry powder was then extracted with boiling ethanol:petroleum ether (skellysolve B) (3:20), the supernatant decanted, and the residue extracted twice more with fresh portions of boiling petroleum ether; the 3 extracts were then combined. All extracts were analyzed for cholesterol by a modification of the Schonheimer-Sperry procedure reported by Nieft and Deuel(9). Total lipids were determined gravimetrically on the petroleum ether extracts of the original tissues.

Results. In Table I are presented the cholesterol and total lipid content of skeletal muscle of dystrophic and non-dystrophic mice. In the dystrophic animals, there is an elevation of muscle cholesterol over that of the normal control; this is in agreement with earlier findings, that a rise in muscle cholesterol obtained in muscular dystrophy(1-5,7). However, in only the dystrophic female mice is there also a significant increase in muscle total lipid.

The plasma cholesterol levels (Table II)

TABLE I. Free Cholesterol and Total Lipid Content of Skeletal Muscle of Dystrophic and Non-dystrophic Mice.

	Group	No. of determinations	Free cholesterol, mg/g wet wt	p*	Total lipid, g/100 g wet wt	p*
♂	Dystrophic	7	1.46 ± .14 †		10.7 ± 5.4 †	
♂	Non-dystrophic	7	.925 ± .028	<.01	7.70 ± .68	.3 > p > .2
♀	Dystrophic	7	1.59 ± .06		11.2 ± 1.2	
♀	Non-dystrophic	6	.954 ± .067	"	6.50 ± .96	.02 > p > .01

* Probability that difference between mean values for dystrophic and non-dystrophic is due to chance.
† ± stand. error.

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TABLE II. Cholesterol Content of Plasma of Dystrophic and Non-dystrophic Mice.

Group	No. of determinations	Cholesterol	
		Free, mg %	Total, mg %
Dystrophic	7	46.8 ± 6.5*	126 ± 20 *
Non-dystrophic	7	43.6 ± 3.4	127 ± 9.5

* ± stand. error.

show no changes in the dystrophic animal as compared with the non-dystrophic mouse. These findings are similar to those obtained in children with muscular dystrophy where the plasma cholesterol is, in fact, slightly lower than normal(10), but are in contrast with results reported from dystrophic rabbits where a hypercholesterolemia obtains(1).

In Table III are the cholesterol and total lipid levels of liver, brain, kidney, lung and heart. In neither male nor female dystrophic mice is there any alteration in the cholesterol and total lipid content of brain and liver. In dystrophic males there is a slight increase in the cholesterol concentration of the kidneys and lungs which is not observed in dystrophic females, whereas in dystrophic females, there is an elevated cholesterol content of the heart. As these changes in the cholesterol content of heart, kidney and lung are of lesser magnitude than those in muscle and are not noted in both sexes, they are probably of doubtful importance. Total lipid values in these tissues are unaffected.

That the only consistent increase in tissue cholesterol in muscular dystrophy is in skeletal muscle indicates that the aberration of cholesterol metabolism in *Dystrophia muscularis* resides in the muscle itself or in some mechanism responsible for lipid transport to or from the muscle. The possibility also exists that the elevated muscle cholesterol arises as a result of degenerated muscle fibers. Experiments are now in progress in an attempt to elucidate the nature of this disturbance in cholesterol metabolism.

Summary. 1) Tissues of mice with hereditary muscular dystrophy and their normal litter-mates have been analyzed for cholesterol and total lipid content. 2) There is a consistent and highly significant increase in cholesterol concentration in the muscle of dystrophic mice of both sexes over that found in the non-dystrophic control animals.

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TABLE III. Cholesterol and Total Lipid Content of Liver, Brain, Kidney, Lung and Heart of Dystrophic and Non-dystrophic Mice.

Tissue	Group	No. of determinations	Cholesterol		Total lipid, g/100 mg wet wt
			Free, mg/g wet wt	Total, mg/g wet wt	
Liver	♂ Dystrophic	7	2.75 ± .15*	3.46 ± .75*	5.20 ± .15*
	♂ Non-dystrophic	7	2.50 ± .06	3.35 ± .10	5.10 ± .15
	♀ Dystrophic	7	2.86 ± .18	4.06 ± .40	5.50 ± .46
	♀ Non-dystrophic	6	2.68 ± .28	3.77 ± .17	5.50 ± .46
Brain	♂ Dystrophic	7	12.5 ± .58	12.5 ± .55	8.04 ± .18
	♂ Non-dystrophic	7	13.6 ± .60	14.2 ± .49	7.46 ± .42
	♀ Dystrophic	7	12.8 ± .36	13.3 ± .11	6.69 ± .43
	♀ Non-dystrophic	6	13.9 ± .36	14.0 ± .12	6.88 ± .20
Kidney	♂ Dystrophic	5	5.49 ± .33	5.51 ± .24	3.95 ± .19
	♂ Non-dystrophic	6	4.39 ± .12	4.51 ± .11	4.41 ± .47
	♀ Dystrophic	7	5.33 ± .22	5.44 ± .17	4.41 ± .45
	♀ Non-dystrophic	8	4.98 ± .13	5.17 ± .15	4.89 ± .40
Lung	♂ Dystrophic	3	6.52 ± .15	6.60 ± .07	7.19 ± 1.2
	♂ Non-dystrophic	4	5.77 ± .10	5.90 ± .10	5.63 ± 1.5
	♀ Dystrophic	3	6.64 ± .19	6.82 ± .37	5.78 ± .18
	♀ Non-dystrophic	3	6.25 ± .33	6.40 ± .35	5.88 ± .34
Heart	♂ Dystrophic	2	1.77	1.91	4.59
	♂ Non-dystrophic	2	1.65	1.78	3.57
	♀ Dystrophic	2	2.11	2.25	4.62
	♀ Non-dystrophic	2	1.76	1.85	4.20

* ± stand. error.

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Effects of Some Steroids on Glycogen Metabolism in Uterus and Skeletal Muscle.* (23756)

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While injections of estrogen result in the deposition of glycogen in the rat uterus(1,2, 3), efforts to produce this response with other steroids such as testosterone, adrenal cortical extracts and progesterone have failed(3,4) with one exception(5). The present study concerns an investigation of (a) ability of certain steroids to elevate uterine glycogen in the spayed rat, (b) effects of combinations of these steroids with estrogen on glycogen deposition and (c) their effects on mobilization of glycogen after epinephrine treatment. Glycogen levels in a representative skeletal muscle were determined concomitantly.

Materials and methods. Virgin female rats weighing 180-220 g were spayed 6-9 days before hormones were administered. In all experiments, the steroids‡ (except estradiol) were injected subcutaneously in 2 mg doses daily for 3 days just prior to autopsy. Estradiol benzoate (either 0.5 or 50 μg doses) was injected subcutaneously 48 hours prior to autopsy. Epinephrine hydrochloride (Parke Davis, Co.) was administered intraperitoneally in doses of 50 μg per 100 g of body weight 1 hour before the rats were sacrificed. Primarily for the study on skeletal muscle glycogen, all rats were fasted 24 hours before autopsy. The rectus femoris muscle and the uterus were removed from anesthetized (Nembutal) rats, rapidly weighed on a torsion balance and digested immediately in hot KOH. The methods used for the digestion and precipitation of the glycogen in these tissues were those previously described(3,6). The anthrone procedure(7) was employed to determine the concentration of glycogen in the tissues and the latter is reported as mg of glucose per 100 g of tissue (wet weight). The data on the uterus are reported in terms of total uterine weight, although most of the glycogen present is in the myometrium(3).

Results. When groups of spayed rats were given injections of the several steroids listed in Table I, the uterine glycogen levels of rats receiving testosterone and desoxycorticosterone were slightly but significantly higher than those of the spayed controls. The inability to demonstrate this effect of testosterone previously(3) was probably due to inadequate hormonal treatment (0.5 mg per day for 2 days). Among the several steroids tested, only testosterone produced a considerable increase in uterine weight similar to that observed in rats after adequate estrogen treatment. Thus, it is possible that the deposition of glycogen in uterine tissue (particu-

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