Effect of Purines and Pyrimidines on Survival and Composition of Residual Muscle of Mice with Hereditary Muscular Dystrophy.* (28228)

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The ability of muscle cells to accumulate potassium from an environment poor in that cation but rich in sodium and chloride appears to be impaired in muscular dystrophy (1). Residual muscle from mice afflicted with hereditary muscular dystrophy contains less potassium(2), more sodium(2) and more chloride(1) than does muscle from normal animals. Dystrophic muscle also contains less adenosine triphosphate (ATP)(3) and phosphorylcreatine (PC)(1), phosphate esters which appear to be intimately related to the cellular mechanism of potassium accumulation and sodium extrusion(4). Dystrophic muscle is more permeable to potassium(5), to soluble proteins such as aldolase(5) and to inulin(6). These observations suggest that a loss of cell membrane integrity occurs in this malady.

The combination of abnormalities in muscular dystrophy is reminiscent of a defect which occurs in the erythrocytes of blood stored for transfusion. During storage at 4° the red blood cell potassium gradually decreases and intracellular sodium increases(7). A slow net hydrolysis of phosphate esters accompanies the cation changes(8). Studies with radiophosphorus suggest that although a slow synthesis of phosphorylated glycolytic intermediates occurs during storage, particularly of 2,3-diphosphoglycerate, there is no synthesis of ATP(9).

When stored blood is transfused, the cation and ATP concentrations are restored to normal within several hours in those erythrocytes which survive(10). Gabrio *et al.*(11) observed that the incubation of stored blood with purine nucleosides *in vitro* resulted in a restoration of glycolysis, reversal of the cation changes, and an increase in ATP and other phosphate esters. In view of the similarity of the storage lesion in red blood cells to the defects in dystrophic muscle, we were prompted to examine the effects of a number of purine and pyrimidine compounds on the life span of dystrophic mice and on a number of constituents of dystrophic muscle.

Materials and methods. Longevity studies were carried out using a mutant of strain 129 mice obtained as weanlings from the Jackson Memorial Laboratory, Bar Harbor, Maine in a fashion already described(1). The compounds tested and dosage used are listed in Table I.

Groups of 5-week-old dystrophic mice were given 25 μg inosine, or 7.5 μg nicotinamide mononucleotide, or a combination of 16 μg hypoxanthine, 7 μg uracil and 2 μg adenosine per g body weight daily by subcutaneous injection. Control dystrophic animals and control parent strain (normal) animals received solvent alone. After 2 weeks of treatment, the animals were sacrificed and muscle electrolytes, non-collagenous nitrogen (NCN), ATP, total adenine nucleotides, PC and free creatine were determined by methods described previously(1).

Results. The effect of administration of purine and pyrimidine compounds on the mean attained age (ST_{50}) of dystrophic mice is summarized in Table I. Dystrophic mice given the free bases hypoxanthine, adenine and uracil had ST_{50}'s of 43, 29 and 27 weeks, respectively, all significantly greater than the ST_{50} of 19 weeks of control dystrophic mice. While administration of inosine, the riboside of hypoxanthine, resulted in a significant increase in mean survival to 28 weeks, the prolongation of ST_{50} of animals given adenosine and uridine was not significant. Dystrophic mice given deoxyadenosine had an ST_{50} of 11 weeks, significantly shorter than that of controls. Significant prolongation of the ST_{50} was also found in animals given 3′, 5′-cyclic adenylic acid (to 31 weeks), diphosphopyri-

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TABLE I. Mean Attained Ages of Dystrophic Mice Treated With Purines and Pyrimidines.

Group	No. of animals	Drug	Dose (mg/g)	ST (50 weeks)
47a	8	Control	Vehicle	19
31	5	Uracil	25	27*
35	7	Hypoxanthine	25	43*
38	9	Adenine	25	29*
51	4	Theobromine	25	24
59	4	Theophylline	25	18
64	11	Nicotinamide	15	17.5
68	7	Uracil Hypoxanthine	8 17	33*
68b	6	Uracil Hypoxanthine	8† 17	40*
5	8	Inosine	20	28*
36	5	"	10‡	28*
12	5	Uridine	25	22
13	4	Cytidine	25	24
14	4	Adenosine	25	25
15	4	Thymidine	25	20
16	4	Deoxyadenosine	25	11*
34	4	Guanosine	25	16
61	5	Inosine monophosphate	40	25
62	5	Adenosine monophosphate	40	25
63	8	Uridine monophosphate	40	24
65	4	Nicotinamide mononucleotide	30	40*
70	4	3′, 5′-cyclic adenylic acid	40	31*
73	4	Diphosphopyridine nucleotide	60	35*

* Significant difference of survival.
† Given on alternate days.
All drugs given thrice weekly except those marked ‡ which were given daily.

dine nucleotide (to 35 weeks) and nicotinamide mononucleotide (to 40 weeks). Administration of nicotinamide to dystrophic mice did not alter their ST_{50}.

In contrast to muscle from parent strain mice, the potassium content of residual muscle from dystrophic mice was lower while sodium and chloride content was increased. Dystrophic mice given inosine or a combination of hypoxanthine, uracil and adenosine showed a partial reversal of muscle electrolyte composition toward normal. Although administration of nicotinamide mononucleotide resulted in a significant prolongation of life span, it had no effect on the abnormal electrolyte content of dystrophic muscle. The data are summarized in Table II. The ATP content of dystrophic muscle was 3.65 ± 0.76 μM/g compared to 5.96 ± 1.04 μM/g in normal muscle. ATP content was even lower in

TABLE II. Some Constituents of Muscle from Dystrophic Mice Treated With Purines and Pyrimidines.

	Dystrophic controls	Treated dystrophic mice: Group 1	Group 2	Group 3	Normal controls
Sodium (meq/kg DFFM)	198 ± 26	157 ± 9	227 ± 19	179 ± 43	85 ± 5
Potassium (meq/kg DFFM)	371 ± 24	385 ± 8	390 ± 15	407 ± 26	434 ± 7
Magnesium (meq/kg DFFM)	85.8 ± 17.9	76.5 ± 11.2	78.9 ± 4.5	78.8 ± 11.4	86.8 ± 11.2
Chloride (meq/kg DFFM)	147 ± 12	103 ± 8	144 ± 6	128 ± 8	78 ± 12
NCN (g/kg DFFM)	112 ± 11	112 ± 6	108 ± 3.5	105 ± 6.9	115 ± 18
Wet weight/DFFM	4.68 ± .11	4.18 ± .52	4.92 ± .15	4.76 ± .20	4.19 ± .04
ATP (μM/g)	3.65 ± .76	2.67 ± .51	2.35 ± .19	4.58 ± 1.76	5.96 ± 1.04
Total adenine nucleotides (μM/g)	4.34	3.57	3.01	5.53	6.26
Free creatine (g/kg)	147 ± 31	134 ± 33	139 ± 29	139 ± 50	165 ± 9
PC (as creatine) (g/kg)	65 ± 16	73 ± 21	73 ± 25	104 ± 27	137 ± 17
K/Na	1.83	2.46	1.72	2.80	5.14

Group 1: 25 μg/g inosine.
" 2: 7.5 " nicotinamide mononucleotide.
" 3: 16 " hypoxanthine.
7 " uracil.
2 " adenosine.

Vehicle: Isotonic saline containing 0.5 mg% chlorbutanol as preservative.
Treatment started in 5-week-old mice. Drugs given daily by subcutaneous injection in 0.01 cc/g vehicle for 2 weeks.
Means ± S.D. of 5 animals are given.

muscle from animals given inosine or nicotinamide mononucleotide. However, ATP in muscle from dystrophic animals given the combination of hypoxanthine, uracil and adenosine was intermediate between that of untreated dystrophic mice and normal animals. PC levels were also lower in muscle from untreated dystrophic mice and dystrophic mice given inosine or nicotinamide mononucleotide than in muscle from normal animals. PC in muscle from dystrophic mice given the combination of hypoxanthine, uracil and adenosine was intermediate.

Discussion. Although dystrophic muscle and stored erythrocytes contain less potassium and more sodium and chlorides than normal, these data indicate that the cause of the ion concentration changes may be different in the two cases. In contrast to stored erythrocytes where incubation with purine nucleosides but not pyrimidine nucleosides nor the free bases restores the electrolyte composition toward normal(11), the ST_{50} of dystrophic mice is prolonged if they are given either pyrimidine or purine nucleosides. The free bases result in even greater prolongation of survival than the nucleosides. In stored erythrocytes, the purine nucleosides are believed to be dissimilated by nucleoside phosphorylase yielding the free base and ribose-1-phosphate which is metabolized further *via* the shunt pathway and it is believed these reactions are coupled to the resynthesis of ATP (11). After long periods of storage, more effective resynthesis of ATP occurs when erythrocytes are incubated with inosine plus small amounts of adenine(12). The resynthesis of ATP is accompanied by restoration of the normal cation concentrations. In muscle and other tissues, however, nucleotides may be formed from free bases by reaction with phosphoribosylpyrophosphate(13).

Simon *et al.*(14) have shown that protein turnover is much more rapid in dystrophic muscle than in normal muscle. The high level of protein synthesis in dystrophic muscle may be accompanied by an increased requirement for RNA. Presently available data on this point are conflicting. Coleman and Ashworth (15) report greater incorporation of labeled glycine into RNA of dystrophic muscle while Girkin, *et al.*(16) found that RNA turnover was not increased in dystrophic muscle.

It is also worth noting that purine and pyrimidine compounds exhibit a positive ionotropic effect on isolated heart preparations (17,18). The positive ionotropic effect and prolonged survival of dystrophic mice are not always accompanied by electrolyte changes. Experiments in progress may explain the mechanism by which these compounds exert their salutary effect in the dystrophic mice.

The prolongation of ST_{50} by administration of nicotinamide mononucleotide or DPN may compensate for a deficiency of tissue DPN or an increased requirement. McCaman(19) studied a number of dehydrogenases in dystrophic muscle and found that while the TPN-linked enzymes were increased, DPN-linked dehydrogenases were decreased.

Summary. Administration of hypoxanthine, adenine, uracil and inosine to dystrophic mice resulted in a significant prolongation of the mean attained age compared to untreated controls, while deoxyadenosine resulted in a significant shortening of life span. Nicotinamide mononucleotide, disphosphopyridine nucleotide and 3′, 5′-cyclic adenylic acid also increased the mean attained age of dystrophic mice significantly. A number of other purine and pyrimidine compounds were only slightly effective or not at all. The low muscle potassium, high sodium and high chloride of dystrophic muscle were partially reversed in dystrophic animals given inosine or a combination of hypoxanthine, uracil and adenosine. In the latter group, the ATP and PC content of muscle was between that of untreated dystrophic mice and normal animals.

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Hormonal Hypercholesterolemia in the Dog: Influence of Niacin, Niacinamide, Benzmalecene, and Cholestyramine Resin. (28229)

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Plasma cholesterol levels of human females fluctuate during the menstrual cycle, and increase considerably during pregnancy(1). The influence of ovarian cyclical changes on cholesterol levels in dogs, has not, to our knowledge, been reported. Canine reproductive physiology differs from the human in that following ovulation in dogs the corpus luteum persists as during the pregnant state, and unmated females commonly exhibit a period of pseudopregnancy.

We report here the occurrence in dogs of high plasma cholesterol level during pseudopregnancy, production of hypercholesterolemia in males and females by injection of progesterone and prolactin, and the influence of niacin, niacinamide, sodium benzmalecene (2), and cholestyramine resin(3,4) on these cholesterol levels.

Methods. Adult beagle dogs, 8 to 15 kg, were used. They were fed a mixture of approximately equal weights of Purina dog meal and Big Time canned dog food in sufficient quantity to maintain body weight. This diet contained, by analysis, 783 mg of cholesterol per average daily portion. Dogs were weighed and blood was drawn once a week. Plasma was prepared by mixing 4.0 ml whole blood with 0.7 ml of citrate-dextrose (ACD) solution, and analyzed for total cholesterol by the procedure of Abell *et al.*(5). Because of dilution with anticoagulant, all reported cholesterol concentrations are about 77% of serum values. Plasma cholesterol concentrations were determined in all dogs for 3 to 10 weeks before experimental regimens were begun. The above analytical procedure did not detect progesterone or its metabolites pregnane-3α-ol-20-one, pregnane-3α-20β-diol, or pregnane-3α-20α-diol.

Progesterone and prolactin† were injected subcutaneously in aqueous suspension 7 times a week. Injections were given twice on Fridays and twice on Mondays, and were omitted over the weekend. Niacin, niacinamide, sodium benzmalecene, and cholestyramine resin, when given, were mixed in the daily diet.

Results. 1. *Pseudopregnancy.* Plasma cholesterol concentrations in 3 female dogs during pseudopregnancy are presented in Table I. Levels increased to approximately

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† Prolactin, containing 3 electrophoretic components (approximately 20 I.U./mg) was prepared in Merck Sharp & Dohme Research Laboratories from sheep pituitaries, using a modification of the method of Cole and Li(11).