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Enzyme Studies in Muscular Dystrophy: IV. Acid Deoxyribonuclease in Nutritional and Hereditary Muscular Dystrophy.* (29016)

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A number of hydrolytic enzymes of muscle, with optimum activity in the acid pH ranges, have been reported to be increased in nutritional muscular dystrophy resulting from Vit. E-deficiency(1·2,3), and in hereditary dystrophy in the mouse(4,5) and chicken (5). It has been suggested that these acid hydrolases are segregated, in an inactive state, in cytoplasmic particles called lysosomes and are released under conditions which cause tissue damage(6). Despite the overall loss of muscle mass, the deoxyribonucleic acid (DNA) content of muscle in nutritional dystrophy and in genetically determined dystrophy of the mouse(7) and chicken(8) is also increased. Since acid deoxyribonuclease (DNase II) is considered to be one of the group of lysosomal enzymes(6), we have investigated its activity in these types of muscle wasting.

Materials and methods. Nutritional muscular dystrophy was produced in young rabbits (600-900 g) by feeding them Diet 11 of Goettsch and Pappenheimer(9) which is deficient in Vit. E and which was treated with 1% ethereal Fe Cl_3. Stripped lard† was used instead of commercial lard. Three times a week, the diet of controls was supplemented by oral administration of 25-50 mg of dl-α-tocopherol in peanut oil. After the appearance of symptoms of Stage II(10) of muscular dystrophy, the animals were sacrificed. Dystrophic mice and their normal littermates, obtained from R. B. Jackson Memorial Laboratory, were 129/Re × C57BL/6 hybrids. The mice were 2-3 months old when sacrificed, and muscle from 2 dystrophic animals usually was pooled for each determination. Dystrophic chickens, raised at this Institute, had been hatched from eggs supplied from the flock maintained by the Dept of Genetics, Univ. of Connecticut.‡ Control chickens were purchased from commercial suppliers. The chickens were approximately 3 months of age when sacrificed.

The method of Coleman(11) was used to determine DNase II activity. Water homogenates of rabbit and mouse thigh and leg muscle and chick breast muscle, prepared in a Waring blendor or Virtis "45" homogenizer, were used as the enzyme source. Under these conditions complete activation of lysosomal enzymes should have been obtained (6). Homogenates were assayed at dilutions where activity varied directly with concentration in an almost linear relationship. Enzyme and substrate blanks were included with each

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TABLE I. DNase II Activity* and NCPN† Content of Dystrophic Muscle.

	mg NCPN per g wet wt	DNase II activity	
		Per g wet wt	Per mg NCPN
Rabbit			
Control(7)‡	32.4 ± 7.3§	6.8 ± 3.4	.21 ± .10
Vit. E-deficient(9)	27.3 ± 4.1	54.7 ± 30.0	2.06 ± 1.12
Chicken			
Control(6)	32.7 ± 3.8	9.1 ± 3.4	.28 ± .12
Dystrophic(7)	26.1 ± 4.3	44.9 ± 19.1	1.72 ± .73
Mouse			
Control(6)	23.4 ± 3.6	38.2 ± 13.5	1.62 ± .48
Dystrophic(5)	18.7 ± .8	178 ± 56	9.6 ± 3.0

* ½ × △ OD 260 per 2 hr.
† Noncollagen protein nitrogen.
‡ No. of determinations.
§ Standard deviation.

assay. All systems were occasionally checked for nonspecific esterase activity by use of incubation mixtures containing inhibitors of both DNase II and alkaline deoxyribonuclease. By using this system DNase activity was suppressed to the same negligible levels in preparations from control and dystrophic animals.

Noncollagen protein nitrogen (NCPN) was determined on alkali digests(12) by use of a biuret method(13) for rabbit and chick muscle, or by the method of Lowry *et al*(14) for mouse muscle.

Results and discussion. In all of the forms of muscle wasting studied, it is evident that DNase II activity (Table I) is significantly increased ($p = <.005$) whether based on wet weight, or NCPN content of tissue. The breast muscle of the dystrophic chicken has 20% less NCPN than that of the control (Table I). In view of the small number of samples in each group, this difference is probably significant ($p = <.02$) and is proportional to the decline in NCPN content of muscle in other forms of dystrophy studied. It would seem, then, that the change in DNase II activity parallels that of other acid hydrolases(1-5) and conforms to the concept of the catabolic function of the lysosomal enzymes(6). The seeming contradiction that both DNase activity and DNA content of dystrophic muscle(7,8) are increased can be most simply explained by the hypothesis of Zalkin, Tappel, and associates(3,5) that the increase in total activities of the lysosomal enzymes is due primarily to infiltration by macrophages possessing high concentrations of these enzymes. This concept is supported by the findings of Gerber *et al*(15) that incorporation of the tritium-labeled, specific DNA precursor, thymidine, is limited to nuclei of macrophages and is not seen in the nuclei of muscle in Vit. E-deficient animals. Thus, most of the increase in DNase II activity and DNA content would be the result of infiltration, and any loss of DNA from the wasted muscle would be masked.

However, other data offer other explanations for the origin and function of increased DNase activity in dystrophic muscle. The histopathology of muscular dystrophies from different causes is basically similar(16); the processes of degeneration, regeneration or attempted regeneration, and an increase in the number of nuclei in the muscle cell are considered by most authorities to be common features. Infiltration by macrophages, however, is regarded as being less significant and its occurrence less consistent. The capacity of dystrophic muscle to regenerate has been demonstrated by incorporation of tritium-labeled thymidine into myotube and muscle-cell nuclei of the genetically dystrophic mouse(17). The patterns of incorporation of thymidine by the muscle in nutritional dystrophy(15) and in hereditary dystrophy(17) may differ not only because of differences in species or causes of muscle wasting, but because of differences in experimental procedures, *i.e.*, in the amounts of tritium-labeled thymidine administered, or in the interval between its administration and the sacrificing

of the animal. Since it has been reported that periodic increases in DNase II activity, as well as that of other acid hydrolases in actively regenerating tissue, are synchronized with the cycles of mitotic division(18), the high levels of DNase II, in dystrophic muscle, may indicate attempts of damaged muscle to regenerate. Perhaps a more important source of increased DNase II activity, as well as that of other hydrolytic enzymes, is an actual increased synthesis of lysosomes by the muscle cell. An increase in number of lysosomes and in activity of acid phosphatase has been reported in the renal papillae of potassium-deficient rats(19).

Summary. Determinations carried out in dystrophic muscle from the mouse and chicken with hereditary dystrophy, and from the Vit. E-deficient rabbit, revealed a 5- to 10-fold increase in activity of acid deoxyribonuclease when compared with controls. Noncollagen protein nitrogen content of dystrophic chicken breast muscle was 20% less than that of controls.

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Reduction of Serum Cholesterol Concentrations by Paromomycin in Patients with Arteriosclerosis.* (29017)

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It has been reported from this laboratory that oral administration of 1.5 to 2 g of neomycin sulfate reduced significantly the concentration of serum cholesterol in patients (1,2,3). Following this finding the effect of oral administration of 15 additional antibacterial drugs on human serum cholesterol levels has been studied(3). Oral para-aminosalicylic acid (8 to 12 g daily) reduced serum cholesterol concentrations comparably to neomycin, and oral administration of kanamycin and chlortetracycline (1 to 2 mg daily) resulted in significant but less marked reduction of serum cholesterol levels(3). All other assayed antibacterial substances had no discernible effect on serum cholesterol(3).

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