which were absorbed only slowly.

Charles and Todd(12) estimated that the barium heparin contained 5 sulfuric acid residues and 2 carboxyl groups/molecule. They believed that the carboxyl groups were unsubstituted. In the current experiments it is probable that only the carboxyl groups are influenced by lowering the pH to 4.0. The state of ionization of the carboxyl groups therefore, may be significant with respect to absorption of heparin.

Our experiments indicate that there is no significant destruction of heparin in the intestinal loop. Approximately 100% of the heparin initially instilled in the gut can be accounted for by estimating amount absorbed. This estimation is based on a metabolic rate of 0.5 μg/kg/minute during the interval that anticoagulant activity was present and measurement of the free heparin remaining in the gut after a 5 to 7 hour experiment. These results indicate that during the experiment when the initial pH in the lumen of intestine was about 4.0, absorption of approximately 10% of instilled heparin took place. During the experiment, however, pH of the luminal content gradually returned toward neutrality, and absorption of drug then ceased.

Summary and conclusion. Sodium heparin is partially absorbed from the lumen of a washed duodenal loop in the dog following addition of acid buffer of pH 4.

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Fat Deposition in Musculature of the Genetically Dystrophic Chicken.* (24975)

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Asmundson and Julian(1) reported that an inherited muscular dystrophy prevented New Hampshire chickens from raising their wings and rising from a flat surface when laid on their backs. The affected birds were homozygous for a recessive autosomal gene (am), and had wider breasts than is normal in this breed. Our exploratory biochemical determinations suggested that this condition in the chicken is similar to certain forms of muscular dystrophy in man(2) characterized histologically by disarrangement of musculature and by large numbers of fat cells. In biochemical characterization of this condition in the chicken, fat deposition was the first major area of interest. When percentage of crude fat in the wet muscle sample was determined, excess fat deposition was associated with physical manifestations of muscular dystrophy, and was a function of age. Further, a sexual dimorphism was discovered, with females generally de-

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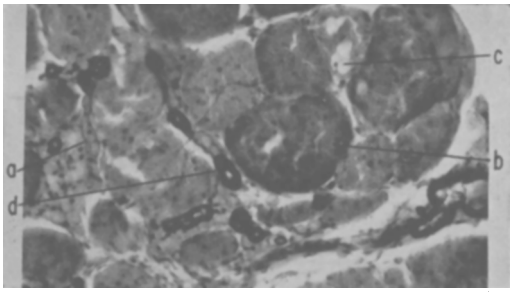

FIG. 1. Superficial pectoral muscle of genetically dystrophic male N.H. chicken, 22 mo of age, fat stain, ×100, containing 27% fat.

positing more fat than males.

Methods. Chicks were brooded and grown to maturity in wire batteries, then placed in laying cages and fed standard ration. Muscle samples to be analyzed were thoroughly minced with scissors, weighed into dry beakers, and dried 18-20 hours in vacuum oven at 100°C and 5 inches of mercury pressure. The dried samples were submerged in petroleum ether (60°-110° B.P.) or toluene and allowed to stand for about one hour. The supernatant was removed and the process was repeated twice, for periods of 3-4 hours, and finally overnight. If necessary, the process was continued (using 2-4 hour periods) until supernatant was clear. The samples were then dried again, by being left overnight under 250-watt heat lamp, followed by 2 hours in oven at 100°C. Fat content is expressed as per cent of wet sample. When muscle size permitted, duplicate samples were assayed. This technic, when compared with one using a fat extraction apparatus (Soxhlet), was equally reproducible (± 10%). To compare histology with fat content, the tissues were fixed in slides in 10% formalin and stained with Oil Red OB stain (Fig. 1). Muscle samples were of 2 origins: 1) from randomly selected birds of the dystrophic strain (NH3A) and of a normal strain of New Hampshires (NH2); and 2) from the anterior superficial pectoral muscle, obtained by serial biopsies at intervals, from 15th through 34th week, from a population consisting initially of 12 NH3A ♂, 9 NH3A ♀, 1 normal ♂, and 2 normal ♀. In several instances, per cent of fat was determined for superficial pectoral, deep pectoral, biceps femoris, and sartorius muscles. In the biopsy series, only the superficial pectoral muscle tissue was analyzed.

Results. The fat content of the superficial pectoral muscle is shown in Table I. Fat content could be considered abnormal only when it exceeded 3%. The deep pectoral muscle appears to mirror the superficial pectoral muscle in fat content (Table II). In normal New Hampshire chicken, fat content of the sartorius and biceps femoris is at least equal to that of pectoral muscles. In the dystrophic bird, the fat content of these 2 muscles does not increase; indeed, in some cases it appears to decrease.

In the superficial pectoral muscle, fat has been seen in 3 forms. These forms are indicated in Fig. 1 as follows: (a) Interfascicular fat is in the form of fat cells that may possibly replace muscle fibers. This has been described in a number of natural and experimental muscular dystrophies of man(2). (b) Small fat droplets can be seen distributed throughout the sarcoplasm of some muscle fibers. The number of droplets varies between fibers; as a result, coloration of individual fibers of a section stained specifically for fat varies markedly. (c) Large intrafibrillar fat vacuoles, possibly smaller droplets coalesced, are situated within the muscle fibers. These are responsible for some vacuolated spaces in routine microscopic preparations. (d) The extremely dark-stained areas are merely air-bubble artifacts.

To date, it has been impossible to demonstrate fat droplets in superficial pectoral muscles of normal New Hampshire chickens. This failure is consistent with the very low fat levels reported above for such muscles.

Discussion. The crude fat content of the superficial pectoral muscle was not significantly different between normal New Hampshires (NH2) and the dystrophic strain of the same breed (NH3A) until sexual maturity (15 to 19 weeks of age). When sex differences were considered in each strain, fat deposition proved significantly different between any 2 groups at the 5% level beginning at 15 weeks old except in normal birds, in which sex differences were significant only at 34 weeks of age. At that age, dystrophic males were significantly different from normal birds only at

TABLE I. % Fat of Wet Sample Weight in Superficial Pectoral Muscle.

	NH3A ♂			Normal ♂			NH3A ♀			Normal ♀		
Age (wk)	No. samples	% fat	E*	No. samples	% fat	E	No. samples	% fat	E	No. samples	% fat	E
4	10	1.7	.2	10	2.5	.3	10	1.9	.2	10	1.1	.3
8	10	2.4	.2				24	1.4	.1			
15†	19	1.4	.2	1	.01	.0	30	2.5	.4	2	.2	.2
19	12	3.6	.7	1	.2	.0	9	8.3	2.1	2	.2	.1
24	11	4.0	.6	1	1.1	.0	11	13.7	2.3	1	.9	.0
34	9	5.1	2.0	1	1.3	.0	10	18.0	4.7	2	2.1	.1
52+	2	6.3	.1				8	25.8	6.4			
78	2	20.6	.5	14	1.4	.4						
84	1	21.0	.0				4	46.6	7.2			

* Stand. error of mean.

† Earliest age at which all results are significantly different from each other (at 5% level) except between Normal ♂ and Normal ♀.

the 10% level, but this can be attributed to a single bird that varied markedly from all other dystrophic males. Fat deposition in dystrophic muscles increased with age.

The data show sexual dimorphism of fat deposition in the superficial pectoral muscle of the dystrophic chicken. This is not evident in the normal chicken, although general lipid metabolism of the bird is influenced by the ovarian estrogenic hormone(3). The data suggest an interactive effect of the endocrine system upon fat deposition in the pectoral muscle of the dystrophic chicken, and ultimately upon severity of the dystrophy syndrome.

Standard errors of means for the data (Table I) reflect a great variation in fat deposition among the dystrophic birds. The crude fat level in certain NH3 males and females was as low as in normal birds, or even lower. A prolonged hypertrophy of muscle tissue is seen in such cases. This suggests that 2 or more strains may be isolated from the existing dystrophic population, based on age at which fat deposition increases markedly.

The bird's inability to rise from a flat surface when laid on its back preceded the symptom of fat deposition by at least 10 weeks.

TABLE II. % Fat of Wet Sample Weight in Muscle.*

Muscle	NH2 (7 ♂)	NH3A (3 ♂)
Sartorius	5.58	.66
Biceps femoris	1.30	.54
Deep pectoral	.74	4.38
Superficial pectoral	1.36	10.08

* Birds were 76-84 wk of age.

Summary. Genetically dystrophic chickens contain significantly more crude fat in the superficial pectoral muscle (% of wet sample) than do normal chickens. An increased fat deposition is noted as early as 15 weeks of age and is associated with physical manifestation of dystrophy resembling certain muscular dystrophies in man. Females deposit more crude fat than males.

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