nism whereby the medullary blood outflow resistance falls during mannitol diuresis remains to be elucidated.

Summary. Measurements were made of the size of the exchangeable albumin pool and the plasma flow rate in the papilla of dogs during hypertonic mannitol diuresis. The former, in ml plasma/100 g tissue, was determined by comparison of labeled albumin in the papilla with that in peripheral plasma. The latter was computed by determining the I^{131} albumin accumulation rate in ml plasma/0.25 min/100 g tissue. The results were compared with similar studies made in hydropenic dogs.

The data are interpreted to support the concept of an extravascular location of albumin in the renal papilla. In addition, it appears that the reported washout of the osmolality gradient and the increased average velocity of plasma flow in this region are probably due to increased reabsorption of fluid into the vasa recta rather than to an increase in inflow of blood to the medulla.

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Serine-Ethanolamine-Phosphate, Taurine and Free Amino Acids of Muscle in Hereditary Muscular Dystrophy of the Chicken.* (28494)

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Muscular dystrophy occurs in New Hampshire chickens homozygous for an autosomal recessive gene(1,2). Birds affected with this disorder cannot raise their wings or right themselves normally when placed on their backs. Marked alterations are found in the large superior pectoral muscle. Muscular degeneration with increasing deposition of fat occurs as the birds grow older(3). Catabolic processes in this, as in other dystrophies, appear to be associated with increased levels of lysosomal enzymes including the cathepsins(4). Possibly related to this enhanced proteolytic activity is a highly characteristic pattern of free amino acids in the dystrophic pectoral muscle. Unusually high levels of taurine and reduced levels of anserine and carnosine have been reported(5). The present study was carried out to define the free amino acid pattern and to characterize a previously unidentified ninhydrin positive component which occurs in considerable amounts in dystrophic pectoral muscle but is present only in traces in normal pectoral muscle. This substance has now been identified as serine-ethanolamine-phosphate.

Methods. Superior pectoral muscles were taken after sacrifice from adult New Hampshire chickens ranging in age from 53 to 137 weeks of age. Females and males with muscular dystrophy were compared with normal females and heterozygous normal male phenotypes.

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Pectoral muscles were also taken from birds at 2, 4 and 6 weeks of age from 3 selected strains (a) normal (line 200), (b) a dystrophic line selected for high muscle fat (line 307), and (c) a dystrophic line selected for low muscle fat (line 308).

Muscle was extracted by homogenizing in 1% picric acid solution in a Servall omnimixer. After centrifuging and filtering the extract was passed over Dowex 2 (Cl) to remove picric acid. Part of the effluent was adjusted to pH 4.7 and passed over Amberlite XE 64 buffered at pH 4.7 to remove basic amino acids and peptides. Since the latter consisted almost entirely of anserine and carnosine it was possible to determine total anserine and carnosine by the difference in the ninhydrin color reaction between the Dowex 2 effluent and the Amberlite XE 64 effluent. Carnosine was determined by the diazo method(6) and anserine then estimated by difference.

Proline was determined by the method of Troll and Lindsley(7) and hydroxyproline by the method of Neuman and Logan(8). Methionine was determined by microbiological assay(19). Other amino acids were determined by the method of Kay *et al*(9) using 2-dimensional paper chromatography in solvent system I followed by II or III.

Solvent systems for paper chromatography:

I Butanol:glacial acetic acid:water (60:15:25 v/v)(10)

II Sec-butanol:tert-butanol:2-butanone:water:diethylamine or 15N NH_4OH (20:20:40:25:1 v/v)(11)

III 95% Ethanol:2-methoxyethanol:15N NH_4OH:water (70:20:5:5 v/v)

IV Phenol:ethanol:water:15N NH_4OH (120:40:40:1 v/v)(10)

V Ethanol:88% formic acid:water (70:10:20 v/v)(13)

Identification of serine-ethanolamine-phosphate. Adult dystrophic muscle contained an unknown ninhydrin reacting substance which could be separated by 2-dimensional paper chromatography from oxidized glutathione by solvent systems I and III or I and IV, but not by I and II. The eluted substance was found on partial hydrolysis in 6N HCl to yield serine, ethanolamine phosphate, ethanolamine and serine phosphate, which were detected by one-dimensional paper chromatography in solvents I, III and IV. After hydrolysis for 24 hours in 6N HCl, serine, ethanolamine and inorganic phosphate were found by chromatography in solvents I, III and IV. In solvent I a third spot appeared corresponding to chloroethylamine as found by Rosenberg and Ennor(13). Hydrolysis in 0.4 N $Ba(OH)_2$ yielded ethanolamine phosphate. The properties of this compound corresponded to those described for serine-ethanolamine-phosphate (SEP) by Roberts and Lowe(12) and Rosenberg and Ennor(13). An extract of dystrophic muscle, prepared as described above, was streaked on numerous Whatman #3 filter papers and chromatographed in solvent system V. The bands corresponding to the unknown compound, located by ultra-violet light and by ninhydrin, were cut out and eluted with distilled water. The solution was fractionated 4 times on Dowex 50 (NH_4+) by elution with distilled water. The methanol addition product was prepared as described by Ennor *et al* for D-SEP(14) and the formamide addition product prepared as described by Jones and Lipkin(15) for L-SEP. Properties of these products (Table I) indicate that the substance is serine-ethanolamine-phosphate.

Results and discussion. Values for free amino acids in pectoral muscle are shown in Table II. Except for hydroxyproline, most of the free amino acids were increased in amount in dystrophic muscle. Carnosine and anserine levels were greatly reduced. This resembles the results found for gastrocnemius of Vit. E deficient rabbits by Tallan (16). The increases in glycine and taurine differ from Tallan's findings. The increases in glutathione are similar to results of Tallan and of Ryerson *et al*(17) for dystrophic rabbits.

The most characteristic components of the dystrophic adult chicken pectoral muscle were the high levels of taurine and SEP. High levels of taurine were found as early as 2 weeks of age (Table III) and are so consistent that they may be used as an indi-

TABLE I. Physical Properties of Serine Ethanolamine Phosphate.

	Isolated L-SEP*	Literature value D-SEP†	Literature value L-SEP‡
m.p. (crystallized from aq. methanol)	143-145°	144-145°	—
m.p. (crystallized from formamide ethanol)	139-141°	—	139-141°
[α] 23.5 D (cryst. aq. methanol)	−19.6° (C = 0.38, H_2O)	+18.0°	
[α] 23.5 D (cryst. from formamide ethanol)	−13.5° (C = 0.5 , H_2O)	—	−15.0°

* Isolated from dystrophic chicken pectoral muscle.
† D-SEP isolated from earthworms (ref. 14).
‡ L-SEP synthetic (ref. 15).

TABLE II. Free Amino Acids of Pectoral Muscle of Adult Chickens.

	♂ Normal phenotype	♀ Normal	♂ Dystrophic	♀ Dystrophic	♂ + ♀ Avg normal	♂ + ♀ Avg dystrophic
No. of birds	4	6	3	3	10	6
% moisture	73.0	73.3	73.6	68.3	73.2	71.2
% fat	2.38	1.47	3.98	10.9	1.86	7.43
% protein	21.8	22.3	19.3	17.3	22.2	18.3
Amino acid			mg/100 g protein*			
Taurine	58	19	668	422	35	544
SEP	23	2	235	95	8	165
Glutathione	57	39	82	119	46	100
Methionine	0.7	0.5	1.0	1.3	0.6	1.2
Valine	15	11	21	22	13	22
Leucines	21	17	25	27	19	26
Glutamic acid	61	58	214	100	59	157
Aspartic acid	8	6	16	12	7	14
Glycine	18	15	48	42	16	46
Serine	18	21	32	46	20	39
B-alanine	25	11	33	53	17	43
Alanine	62	36	72	108	46	90
Threonine	31	34	57	35	33	46
Glutamine	54	45	123	120	48	122
Hydroxyproline	10	4	6	9	6	8
Proline	19	14	22	34	16	28
Anserine	2818	3780	937	1380	3391	1158
Carnosine	914	1297	328	828	1143	578

* "Protein" = nitrogen × 6.25.

cator of muscular dystrophy.

SEP did not appear in pectoral muscle of either normal or dystrophic chicks at 2 weeks of age. In the dystrophic chicks an unknown ninhydrin positive component, possibly a precursor of SEP, was present at 2 weeks of age. At 4 weeks of age both SEP and the unknown compound appeared, but by 6 weeks of age the unknown compound was either absent or present in trace amounts while SEP was present in all dystrophic pectoral muscles (Table III). SEP occurred in lower amounts of strain 308 (low fat line) than in strain 307 (high fat line) suggesting a relationship to fat metabolism, possibly with phospholipids. A sex difference in the distribution of both taurine and SEP may be indicated by the results in Table III.

The function of SEP is not known. It has been isolated from turtles(12), earthworms (where it occurs as D-SEP)(14), and its distribution in the tissues of the chicken has been studied(20). It is reported to have been found mainly in birds, reptiles, and amphibians(20).

The low levels of anserine and carnosine in muscular dystrophy are of interest since anserine does not decrease in muscle of chicks

TABLE III. Muscle Components of Young Chicks.

Strain*	Sex	No. birds	H_2O (%)	Protein (%)	Fat (%)	Taurine	Glutathione	SEP	Unknown as leucine
						(mg/100 g protein)			
				2-week-old birds					
Normal	♂	5	73.7	22.8	2.48	67	39	0	0
"	♀	5	72.9	21.4	2.36	58	15	0	0
307	♂	5	76.3	19.5	3.94	147	73	0	132
"	♀	5	75.5	18.4	4.46	145	95	0	101
308	♂	4	76.2	19.5	1.80	162	197	0	128
"	♀	4	75.3	19.2	2.43	137	132	0	128
				4-week-old birds					
Normal	♂	3	74.1	20.3	1.54	40	23	0	0
"	♀	3	75.9	23.1	2.38	30	20	0	0
307	♂	3	76.9	18.5	4.50	397	128	112	6
"	♀	3	77.5	18.5	4.16	279	114	21	6
				6-week-old birds					
Normal	♂	5	73.1	23.8	1.63	23	56	0	0
"	♀	5	72.4	23.9	1.01	18	28	0	0
307	♂	5	76.8	18.5	4.44	534	126	60	0
"	♀	5	75.5	19.7	4.46	354	162	50	0
308	♂	5	76.4	19.6	1.96	433	108	30	0
"	♀	5	76.1	19.5	1.85	508	108	17	0

* 307 high fat dystrophic line—308 low fat dystrophic line.

on a histidine-free diet, while carnosine falls to very low levels on such diets(18). The effects in muscular dystrophy may be related to increases in proteolytic enzymes released from lysosomes as suggested by Tappel *et al* (4), but the cause of this remains undetermined.

Summary. Carnosine and anserine were decreased in pectoral muscle of chickens with hereditary muscular dystrophy, while levels of other free amino acids except hydroxyproline were increased. The increases in levels of taurine and serine-ethanolamine-phosphate (SEP) were more than 10-fold in adult dystrophic chickens. These two substances could be used as diagnostic signs of muscular dystrophy. In the 2-week-old dystrophic chicks SEP was preceded by an unknown compound which disappeared as SEP began to appear.

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ATPase, Myokinase, 5'Adenylic Acid Deaminase Activity and Syneresis of the Myofibrills of the Dystrophic Mouse.* (28495)

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Previous work of Fischer(1), Aloisi *et al* (2,3,4) and Feuer and Frigyes(5) has shown qualitative and quantitative differences in the contractile proteins of the rabbit in denervation, E-avitaminosis and nutritional atrophy. Sandow and Burst(6) found pronounced changes in the contractility and Zymaris *et al* (7) in the nucleotide composition of the muscle of the dystrophic mouse(8). Such findings led us to investigate the activity of certain enzymes immediately involved in the ATP† metabolism of the dystrophic mouse. Syneresis (superprecipitation) of the myofibrills was used to study contraction. This model appeared to be most suitable because of its high actomyosin content and its structural organization(9).

Materials and methods. Myofibrillar suspension was prepared as described previously (10). Myokinase was prepared according to Colowick(11). The preparation of myosin-B was carried out as described by Mommaerts (12). ATP, ADP, AMP and ITP were purchased from the Sigma Co. of St. Louis, Mo. All other reagents were of reagent grade.

ATPase determinations were carried out at 37°C for 5 minutes and were assayed as previously published(10). The composition of the individual incubation mixtures is described in detail in Table I. Myokinase activity was determined according to Colowick (11), using rabbit myosin-B as the ATPase. Deaminase activity of the myofibrills was assayed with the Conway microdiffusion method(13) as described earlier(14). The mean deviation (an average of deviations from the mean) between replicate analysis of the same batch was within 5% of all enzymes studied. The mean deviation between batches is listed in the respective tables. The syneresis of the myofibrills(15) was investigated at 23°C in a 0.05 M, pH 7.5 buffer. The results were evaluated 3 minutes after addition of ATP by judging the amount of the shrunken, clumped myofibrills precipitated at the bottom of the test tube. Noncollagenous nitrogen was extracted according to Lilienthal *et al*(16). This was used as a correction factor in calculation of the enzyme activity. Protein concentration was determined by a micro-Kjeldhal method.

Results. The ATPase and ITPase activity of the myofibrills of the dystrophic mouse showed no significant difference from the normal control, irrespective of the kind of activator and the ionic strength and pH of the incubation mixture employed (Table I). The ATPase activity of the dystrophic myofibrills, however, showed a more rapid decrease during storage at plus 1-3°C than did the normal

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† In this paper the following abbreviations have been used. ATP: adenosinetriphosphate, ADP: adenosinediphosphate, AMP-5': adenosine-5-monophosphate, AMP 2'3': a mixture of adenosine-2-monophosphate and adenosine 3 monophosphate, ITP: inosinetriphosphate, ATPase: adenosine triphosphatase, ITPase: inosinetriphosphatase μM P_i/mg/min: micromoles of inorganic phosphate split by 1 mg of protein in 1 minute, BAL: British anti-lewisite.

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