crease the urinary content of copper in patients receiving the drug(15). Ephedrine sulfate and methoxamine were weakly active in the gamma globulin system in competing with cysteine for copper but have not been reported to induce rheumatic disease.

The observations here reported suggest that a drug like hydralazine which induces rheumatic like disease, activates the sulfhydryl group in the presence of copper and may thereby facilitate sulfhydryl-disulfide exchange between protein molecules causing protein denaturation.

Summary. Fifty-one compounds were studied for their ability to compete with the sulfhydryl group for cupric ions. The sulfhydryl-disulfide interchange reactions occurring during the reaction between cysteine and bis(p-nitrophenyl)disulfide and during the course of the denaturation of human serum gamma globulin by heat were used as models for this reaction. Hydralazine, a compound which has been reported to induce a disease resembling systemic lupus erythematosus was exceptionally active in reactivating the sulfhydryl-disulfide interchange reaction when this reaction was studied in the presence of cupric ion.

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Free Amino Acids of Developing Skeletal Musculature of Normal and Genetically Dystrophic Chickens.* (30109)

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Muscular dystrophy, inherited as a recessive trait in New Hampshire chickens, is manifested by the inability of the birds to raise their wings and to perform the normal righting reflex when placed on their backs(1). Hypertrophy of the superior pectoral muscle occurs followed in some birds by a loss of muscle fibers. The breast muscle of dystrophic chickens has a higher fat(2) and free amino acid content(3), a higher water to nitrogen ratio(3) and a higher lysosomal enzyme activity(4) than that of normal chickens. Carnosine and anserine levels are lower than in normal birds(3).

Elevated levels of taurine and serine ethanolamine phosphate (SEP) are especially characteristic of dystrophic breast muscle(3). In 2 lines of dystrophic birds selected for low and high fat content SEP appeared in the breast muscles no earlier than 4 weeks of age. It was not detected in the breast muscle of 2-, 4-, and 6-week-old normal chicks. An unidentified ninhydrin-positive compound (hereafter referred to as CX) was present

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TABLE I. Characteristics of Chick Breast Muscle.
Normal line 200 and dystrophic line 304 average of 6 to 12 samples.

Age	Line	% H_2O	% protein	% fat	Mg/100 g protein			
					Taurine	GSH	CX*	SEP
2 week	200	75.2	23.2	—	47	75	7	0
	304	78.1	20.2	—	131	176	89	24
4 "	200	74.1	21.7	1.96	35	22	0	0
	304	76.1	18.4	4.84	386	149	10	68
6 "	200	72.8	23.9	1.38	20	42	0	0
	304	75.3	20.3	4.02	457	170	0	91

* As leucine.

in the breast muscles of the 2 dystrophic, but not the normal lines at 2 weeks of age. It disappeared from the tissues concomitant with the appearance of SEP.

The above mentioned findings raised the question of whether or not embryonic muscle from normal chickens differed in composition from dystrophic muscle and of whether or not other dystrophic lines would show the same changes in juvenile muscle composition as did the two lines selected for low and high fat content.

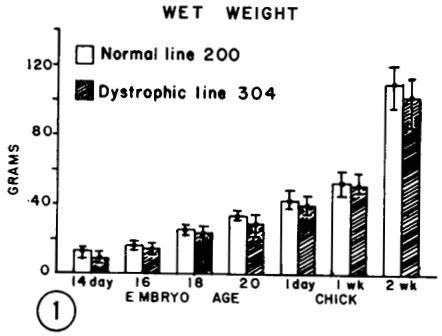
Materials and methods. Embryos and young chicks from 2 lines of New Hampshire chickens were studied. These were line 200, the normal line, and line 304, a line selected for early onset of the disorder. Unpublished data of Asmundson indicate that 90% of the chicks in line 304 were dystrophic before 3 weeks of age as measured by their failure to right themselves when laid on their backs.

Fertile eggs were incubated in Jamesway incubators. One-day-old chicks were not fed, but were given water before being sacrificed. Chicks were fed a stock diet and kept in wire cages until sacrificed at 2, 4, and 6 weeks of age.

Eggs were removed from the incubator at 14, 16, 17, 18, and 20 days of incubation and stored up to several hours in a constant temperature chamber at 38°C. Embryos were removed from eggs and weighed without yolk sacs and embryonic membranes. Leg and breast muscle tissues were dissected out and placed on dry ice. Samples were taken for moisture and Kjeldahl nitrogen determinations. Total protein was estimated as nitrogen $\times$ 6.25. Thirty mg of picric acid and usually 1.0 ml of water were added per gram of wet weight of muscle and the tissues were homogenized in a Servall Omnimixer chilled in an ice bath. Homogenates were filtered and stored in the frozen state.

Aqueous picric acid extracts were chromatographed by the method of Peterson *et al* (3), using 2 dimensional paper chromatography. The solvent systems used for quantitative determination of the amino acids were I. 95% ethanol : 2-methoxy-ethanol : 15 N NH_4OH : water (70:20:5:5 v/v) followed by II. butanol : glacial acetic acid : water (60:15:25 v/v). The chromatograms were dipped in a ninhydrin solution and heated for 30 minutes at 60°C. The ninhydrin-positive spots were cut out, eluted with water and the optical density of the colored solutions determined.

Results. Two- to six-week-old chicks. Differences in composition between the breast muscles of normal line 200 and dystrophic line 304 chicks of 2, 4 and 6 weeks of age are shown in Table I. The data indicate that breast muscles from the dystrophic line 304 had consistently higher moisture, lower protein and higher fat contents than did those from the normal line. Taurine and glutathione concentrations were also higher in dystrophic than they were in normal tissues. Taurine levels increased with the age of the dystrophic chicks. The unidentified ninhydrin-positive compound CX was present in the breast muscles of 2-week-old dystrophic chicks. The concentration of CX fell to a relatively low level in the breast muscles of chicks of 4 weeks of age, and was not present in the muscles of 6-week-old birds. A low but detectable amount of CX was found in the breast muscle of normal 2-week-old chicks.



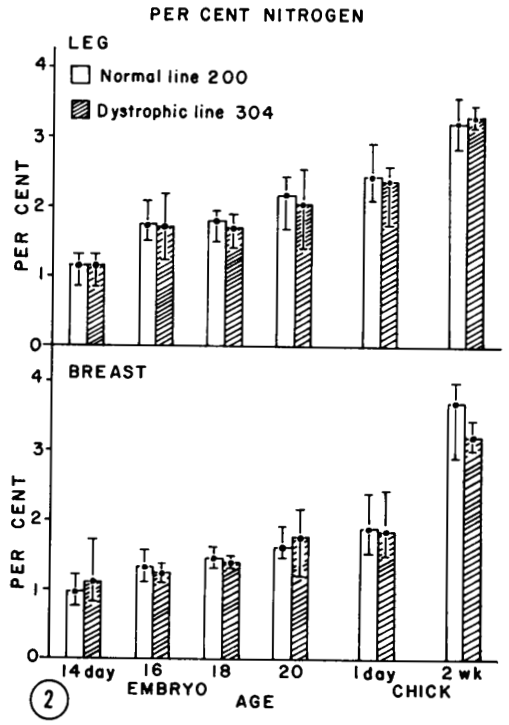


FIG. 1. Wet weight of normal and dystrophic embryos and young chicks. Points represent averages of 5 to 12 samples. Bars denote range.

FIG. 2. Nitrogen content of leg and breast muscles of normal and dystrophic embryos and chicks. Points represent averages of 5 to 12 muscles. Bars denote range.

SEP was present in the breast muscles of 2-week-old dystrophic chicks and increased to almost 4 times its original value by the time the chicks were 6 weeks old. SEP could not be detected in the breast muscles of normal line 200 birds.

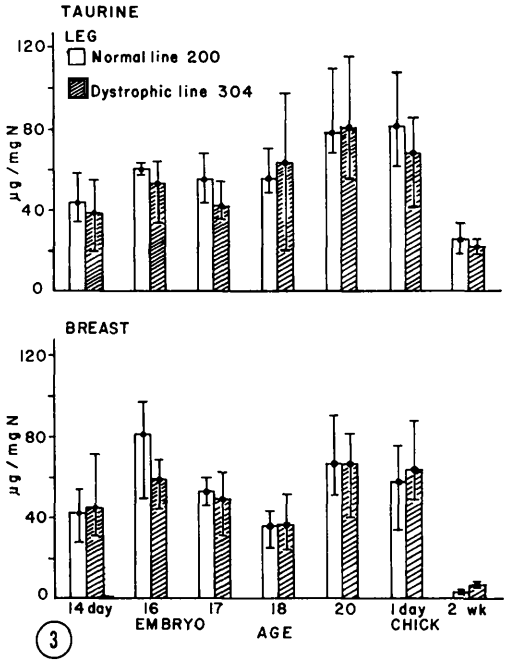
Growth of late embryos and young chicks. The increase in wet weight of line 200 and line 304 embryos and young chicks from 14 days of incubation to a post-hatching age of 2 weeks is shown in Fig. 1. Although there were no large differences between the wet weights of the 2 lines, values for line 304 were somewhat lower than those for line 200. Fig. 2 shows the mean nitrogen content of the leg and breast muscles of embryos and young chicks from the 2 lines. Nitrogen content of the embryonic leg muscles tended to be slightly higher than that of the breast muscles throughout the last week of embryonic life. However, nitrogen content of the breast muscles was greater than that of the leg muscles after the first 2 weeks of post-hatch life. The nitrogen contents of the leg muscles of line 200 and line 304 birds were essentially the same; nitrogen contents of the muscles did not become obviously different until the chicks were 2 weeks old.

Examination of the chromatograms revealed no outstanding differences in the free amino acid patterns of the leg and breast muscles of the dystrophic and normal lines until after the chicks hatched. Indeed, the embryonic tissues differed more from the chick tissues than they differed among themselves. The data in Fig. 3 illustrate these findings for a single amino acid, taurine. The taurine levels of all the embryonic muscles were considerably higher than those of the 2-week-old chicks and the dystrophic breast muscles did not contain significantly more taurine than their normal counterparts until the chicks were 2 weeks old.

Other major differences between the free amino acid patterns of the embryonic and chick skeletal muscles are indicated by the following observations: SEP did not appear in the embryonic muscle samples; anserine and carnosine were first detected in the muscles of one-day-old chicks; phosphoethanolamine decreased steadily during the last week of embryonic life, and the chromatograms of embryonic but not chick leg and breast muscles contained large amounts of compounds in the region occupied by the basic amino acids.

Most of the soluble ninhydrin-positive compounds either remained unchanged, fluctuated or declined during the last week of embryonic life. Only one compound increased in concentration during this period; this was com-

pound CX. Fig. 4 shows the changes in CX concentration. It was present in both embryonic leg and breast muscles of the 2 genetic lines and its concentration increased from about the 18th day of incubation to at least the first day post-hatch. The data indicate that the level of CX was consistently higher in breast than it was in leg muscle. CX decreased in concentration during the first 2 weeks of post-hatch life so that by 2 weeks of age it had almost disappeared in all but the dystrophic breast muscles.



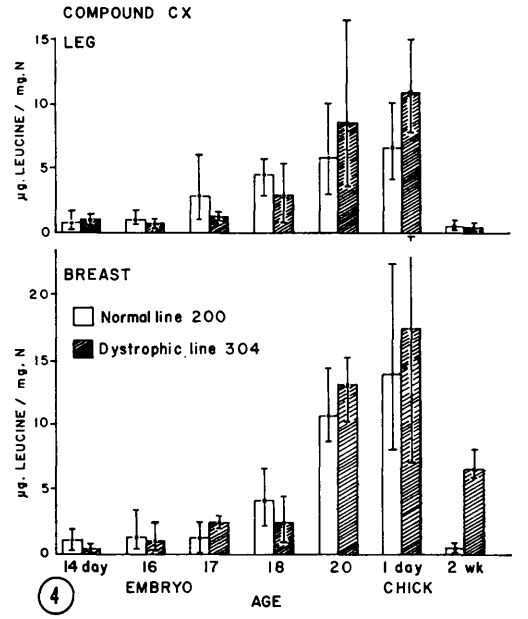


FIG. 3. Taurine content of leg and breast muscles of normal and dystrophic embryos and chicks. Points represent averages of at least 5 samples. Bars denote range.

FIG. 4. CX content of leg and breast muscles of normal and dystrophic embryos and chicks expressed as μg leucine/mg N. Points represent averages of at least 5 samples. Bars denote range.

Discussion. The results of this study indicate that the breast muscles of dystrophic chicks retain some of the properties of embryonic breast muscles. The strongest evidence for this statement is the fact that the ninhydrin-positive compound CX, previously shown to be a component of 2- and 4-week-old dystrophic breast muscle(3) is a characteristic constituent of the embryonic leg and breast muscles from both normal and dystrophic lines. In addition, the data presented here, as well as other studies show that embryonic tissue(5,6) and dystrophic tissue(3) contain relatively high concentrations of taurine and other ninhydrin-positive compounds.

In addition to the above data work from 2 other laboratories suggests that dystrophic chicken breast muscle retains some embryonic traits. Kaplan and Cahn(7) found that the lactic dehydrogenase (LDH) of dystrophic chicken breast muscle resembled more closely the LDH of embryonic breast muscle than it did that of normal chicken breast muscle. Also, Herrmann *et al*(8) found "embryonic culture patterns" with explants of dystrophic but not those of normal chicken pectoral muscle.

The moisture, protein, fat, taurine, glutathione and compound CX contents of the breast muscles of dystrophic line 304 chicks were similar to those obtained from the 2 dystrophic lines studied earlier by Peterson *et al*(3). Thus, selection for fat content and time of onset of dystrophy could not be related to the chemical characteristics which distinguish 'dystrophic' from 'normal' chick breast muscle.

Generally speaking, the 4 embryonic muscles studied, normal line 200 leg and breast muscles and dystrophic line 304 leg and breast muscles, had similar distributions of

water soluble ninhydrin-positive substances. The free amino acid patterns characteristic for the specific tissues were not established until after the chicks hatched.

Even though the 4 embryonic muscles were similar in composition, their characteristics changed with time, indicating that new enzyme systems had been activated. Compound CX increased in amount at the 18th day of embryo incubation; anserine and carnosine, as previously noted by Lowe(6) were first detected in the skeletal muscles of day-old chicks, and SEP appeared in the dystrophic breast muscles sometime during the first 2 weeks of post-embryonic life. Such temporal and genetic markers of muscle development may prove useful in future studies of the factors which regulate the metabolic changes associated with embryonic growth and differentiation.

Summary. The free amino acid patterns of leg and breast muscles were studied during the development of normal and dystrophic chickens from the 14-day-old embryo to the 2-week-old chick. Although these patterns changed with time little difference was found between muscles or strains until after hatching. An unidentified ninhydrin positive substance (CX) was present at high levels in the muscles of 18- to 20-day-old embryos and day-old chicks; CX rapidly declined in all but the dystrophic breast muscle by the time the chicks were 2 weeks of age. Its subsequent disappearance was accompanied by the appearance of serine ethanolamine phosphate, which occurs characteristically in dystrophic breast muscle. Taurine, also occurring at high levels in the embryo, remained high in dystrophic chick breast muscle. The results indicate that dystrophic chick muscle retains some embryonic properties and that this form of muscular dystrophy involves changes in muscle development.

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Inflammatory Effects of Pyrogenic Steroids in Animals. (30110)

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Several 5β-H steroids produce intense fever and inflammation when injected into man (1-3). The pyrogenicity of neutral steroids, such as etiocholanolone and 11-ketopregnanolone, is highly species-specific(4). This investigation was undertaken to determine whether the pyrogenicity of the acidic steroid lithocholic acid was similarly species-specific, and to examine the possible inflammatory properties of the 3 steroids in experimental animals.

Methods. Studies on inflammation were done on unrestrained animals. After shaving the abdomen, steroid solutions (20 mg/ml in propylene glycol) were injected subcutaneously on one side of the abdomen, and an equal volume of solvent vehicle on the other. Injection sites were observed for gross

* Operated by University of Chicago for U. S. Atomic Energy Commission.