tivity of the antigen binding tests for IgG, these results, substantiated by indications of slowly sedimenting antibody activity in spleen and lymph node lysate from 5-day BGG stimulated animals, are indicative of the early synthesis of low molecular weight antibody. These and other recent data (15–17) suggest that a clear-cut sequential synthesis of 19s IgM and 7s IgG is not a consistent feature of the early antibody response.

Summary. Cellular production of antibody to several proteins was studied using antigen-coupled erythrocytes in a modified passive hemolytic plaque assay. The kinetics of plaque formation correlated well with serum 19s IgM antibody, although a modified antigen binding test indicated early concomitant synthesis of 7s IgG antibody. Results of plaque assays for several protein antigens are presented.

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Occurrence of Cystathionine in Breast Muscle of Genetically Dystrophic Chicks* (32745)

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During a study of the free amino acids in breast muscle of chicks with hereditary muscular dystrophy (1), it was observed that an unknown ninhydrin positive constituent was present in dystrophic pectoralis muscle at 2 weeks of age but absent in normal pectoralis muscle. This substance declined sharply by 4 weeks of age and was no longer present at 6 weeks of age. In a further (2) study it was observed that the unknown compound was present in small amounts in both normal and dystrophic embryonic chick leg and breast muscle. It began to increase by the eighteenth day of incubation and had reached high levels just after hatching. Thereafter it declined in leg muscle from normal and dystrophic chicks and in normal breast muscle but remained high in dystrophic breast muscle so that by 2 weeks of age it had almost disappeared from all but dystrophic breast muscle.

The present paper describes the isolation and characterization of this substance.

Experimental

Solvent systems for paper chromatography. I. 95% ethanol:2 methoxy ethanol:15 *N* NH_4OH:H_2O (70:20:5:5 v/v) (1); II. *n*-butanol:glacial acetic acid:water (60:15:25 v/v) (3); III. methanol:2 ethoxyethanol:

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H_2O:15 *N* NH_4OH (40:40:15:5); IV. phenol:ethanol:15 *N* NH_4OH:H_2O (120:40:1:40 v/v) (3); V. butanol:pyridine:H_2O (1:1:1 v/v) (3); and VI. *tert*-butanol:formic acid:water (14:3:3 v/v) (4).

Characteristics of unknown compound. Protein free extracts of breast muscles from 2-week-old dystrophic chicks were prepared as previously described (1). Two dimensional chromatographs on Whatman no. 1 filter paper were obtained with solvent systems no. I and II. The unknown substance gave a positive test for sulfur with the reagent of Winegard *et al.* (5). No reaction was obtained with the nitroprusside reagent of Toennies and Kolb (6), indicating the absence of disulfide or sulfhydryl sulfur. Chromatographic characteristics of the unknown suggested that it might be cystathionine. When cystathionine was added to the muscle extracts and two dimensional paper chromatograms run with solvents I, III, IV, V, or VI in the first direction and solvent II in the second direction, the unknown substance and cystathionine appeared in the same position on the chromatograms.

Separation of unknown from embryos. Heads and feet were removed from 20-day-old dystrophic embryos. They were extracted several times by homogenizing with water at 90°C in a Servall omnimixer. Insoluble material was removed by centrifugation and filtration. Thirty mg of picric acid per gm of embryo was added to the filtrate, which was blended in the omnimixer. The precipitate was centrifuged and filtered and picric acid removed with Dowex 2x8 (Cl^-). The effluent was adjusted to pH 6.5 and concentrated in a rotary evaporator and fractionated as described below; each step was checked by paper chromatography. The samples were streaked on Whatman no. 3 filter paper and developed with solvent system III followed by solvent system II in the same direction. Bands containing the unknown were eluted with water and these solutions were chromatographed on Dowex 50 × 8 (NH_4^+) (200–400 mesh) and eluted with water. The eluate was then poured over a Dowex 50 × 8 column prepared with ammonium formate buffer at pH 3.3 (7) and eluted with the same buffer. The eluate was again chromatographed on Whatman no. 3 filter paper and eluted with water. The eluate was again chromatographed on Dowex 50 (NH_4^+) and eluted with water. A small amount of crystalline material was obtained by concentrating and chilling the solution.

When this material was dissolved in water and chromatographed in solvents I, III, IV, and V followed by solvent II, it had chromatographic characteristics identical to those of authentic L-cystathionine and appeared to be free of impurities. Insufficient material was available for further characterization.

Separation of unknown from dystrophic breast muscle. Protein free extracts were prepared from 320 gm of pooled breast muscle from 2-week-old dystrophic chicks in a manner similar to that described above for embryonic tissue. The unknown was fractionated from the extract as described below; each step was verified by paper chromatography.

The extract was adjusted to pH 3.3 with NaOH and chromatographed on Dowex 50 prepared with ammonium formate buffer (pH 3.3) and eluted with the same buffer. Fractions containing the unknown were pooled, adjusted to pH 2.8, and poured on a Dowex 50 column prepared with ammonium formate buffer at pH 3, and eluted with the same buffer solution. Fractions containing the unknown were collected and deionized on a Dowex 50 (H^+) column by the method of Carsten (8). Final purification was effected by several passes over Dowex 50 (NH_4^+) using water as eluant. The solution was concentrated and chilled in ice. The crystals which formed were collected, washed with ice water, and dried over anhydrous calcium sulfate in vacuum. The yield was approximately 15 mg.

Determination of optical rotation in a Zeiss polarimeter resulted in the value $[\alpha]_D^{25}$ + 25° ± 2.5 (0.8% in *N* HCl). Literature values for L+cystathionine are $[\alpha]_D^{25}$ + 26.4° (7), $[\alpha]_D^{25}$ + 26° ± 2 (9), $[\alpha]_D^{20}$ + 23.9° (10).

Infrared absorption spectra of the isolated compound and of a sample of authentic L+

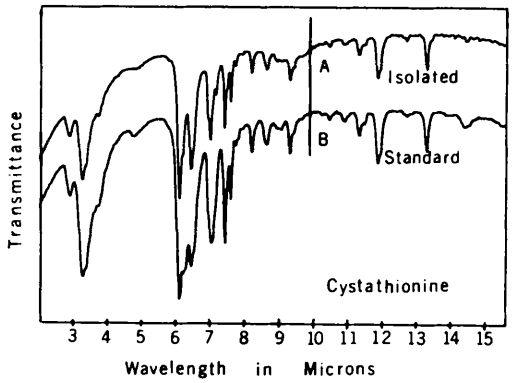

FIG. 1. Infrared absorption of isolated *L*-cystathionine in KBr compared with standard of *L*-cystathionine.

cystathionine (K and K Laboratories) are shown in Fig. 1.

The isolated substance and its oxidation products with hydrogen peroxide–molybdate treatment (11) had identical chromatographic properties on paper to those of L-cystathionine when tested by one dimensional chromatography in solvents II and VI and by two dimensional chromatography in solvent systems II and V; VI and II; and VI and V. Two principal ninhydrin-reacting oxidation products were obtained. One of these was identical with the principal peroxide oxidation product of homocystine.

The properties of the isolated material indicate that the substance present in dystrophic breast muscle is cystathionine.

Discussion. The occurrence of considerable amounts of cystathionine in breast muscle of dystrophic chickens at 2 weeks after hatching when it has almost disappeared from breast muscle of normal chicks is further evidence for the persistence of embryonic characteristics in the dystrophic muscle. Similar observations have been reported for taurine (1,2). Kaplan and Cahn (12) have found that the lactic dehydrogenase isozymes of dystrophic chicken breast muscle are more like those of embryonic muscle than that of normal breast muscle. Additional evidence for the persistence of embryonic patterns in dystrophic muscle is to be found in the data of Cosmos (13) on phosphorylase; and Weinstock (14) on DNA, deoxyribonuclease, and cathepsins.

These data offer support for the concept that dystrophic muscle shows characteristically retarded or incomplete development. In some instances the juvenile characteristics are maintained in the dystrophic muscle long after the birds have matured chronologically (1, 14).

Summary. A ninhydrin positive compound present in embryonic chick muscle and in dystrophic chick breast muscle at 2 weeks after hatching was shown to be cystathionine. Cystathionine is characteristically present in embryonic muscle and its persistence in dystrophic muscle several weeks after hatching when it has disappeared from normal muscle is further evidence for retarded development of dystrophic muscle.

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