Studies on Amino Acids in Embryonic Tissue. I. L-Lanthionine, a Naturally Occurring Amino Acid in the Chick Embryo*

N. H. Sloane and Karl G. Untch†

ABSTRACT: It is demonstrated that L-lanthionine is a naturally occurring amino acid. It was found in the free amino acid pool of the chick embryo and was identified by spectroscopic and authentic sample comparisons. Evidence has also been obtained which indicates that L-lanthionine is a constituent of chick embryo protein. Both L- and *meso*-lanthionine were isolated from heat-treated, acid-hydrolyzed chick embryos. The isolation of *meso*-lanthionine from the heat-treated embryos can be explained on chemical grounds.

Horn *et al.* (1941) reported the presence of a mixture of lanthionine isomers in the acid hydrolysate of wool that had been pretreated with alkali. These investigators isolated two optically inactive isomers of lanthionine that differed in solubility properties. Synthetic studies by du Vigneaud and Brown (1941) and Brown and du Vigneaud (1941) showed that the *meso* isomer could be crystallized free from the DL racemic mixture. Subsequently, Horn and Jones (1941), du Vigneaud *et al.* (1941), and Horn *et al.* (1942a,b) demonstrated that alkaline treatment of other proteins followed by acid hydrolysis also resulted in the formation of a mixture of lanthionine isomers. These investigators concluded that these lanthionine isomers were artifacts formed by the action of alkali on the proteins. In a later investigation, Cuthbertson and Phillips (1945) found no detectable amounts of lanthionine in acid hydrolysates of wool which had not been pretreated with alkali and concluded that lanthionine was not present naturally in wool. These workers offered a mechanistic explanation for the presence of lanthionine in acid hydrolysates of wool samples that had been pretreated with alkali, *i.e.*, lanthionine isomers are formed after base-catalyzed chemical cleavage of the disulfide bond of L-cystine, followed by a condensation of the postulated resulting L-cysteine and α-aminoacrylic acid to yield, theoretically, equal amounts of *meso*- and L-lanthionine. The fact that these investigators obtained optically inactive lanthionine (DL), the authors believe, could have been due to racemization of the L-cysteine or L-lanthionine under the alkaline conditions employed in their experiments. The authors have found that L-lanthionine does racemize in an alkaline solution (see below).

In this paper the authors prove that L-lanthionine is a naturally occurring amino acid. It has been isolated from the free amino acid pool of the chick embryo. Evidence is also presented which (a) indicates that L-lanthionine is a constituent of protein in the chick embryo, and (b) demonstrates that *both* L- and *meso*-lanthionine can be isolated from heat-treated chick embryos. This latter observation, obtained under the more drastic conditions, is best interpreted as being the result of a chemical transformation of cystine. Since it has already been shown that lanthionine can be isolated as an artifact, it was not only necessary to isolate optically active lanthionine, but also to demonstrate the absence of the *meso* isomer, in order to prove that L-lanthionine occurs naturally in chick embryo.

Results and Discussion

Isolation of L-Lanthionine from the Amino Acid Pool of Acid-Treated Chick Embryo Extract. In order to prove that L-lanthionine occurs naturally in the chick embryo, it was essential to demonstrate the presence of the L isomer of the amino acid in the complete absence of the *meso* compound. As we have noted above, the presence of *meso*-lanthionine in any mixture indicates that lanthionine isomers can arise chemically. Thus to prove that L-lanthionine is present naturally in the chick embryo, the nonheated homogenate (after centrifugation) was adjusted to pH 3.5 to remove protein and then subjected to ion-exchange chromatography (see Experimental Section for details). Amino acid analysis of the 6 N HCl eluate of the Dowex 50 resin showed (a) the presence of a ninhydrin-reactive compound that corresponded in elution characteristics to L-lanthionine[1] [Figure 1, curve D (A144)], and (b) the absence of ninhydrin-positive material in the area corresponding to *meso*-lanthionine (Figure 2, curve B). The ninhydrin-positive material of Figure 1, curve D was isolated and crystallized as described (see Experimental Section): its amino acid analysis is shown in

*From the Department of Biochemistry, University of Tennessee School of Basic Medical Sciences, Memphis, Tennessee, and Mellon Institute, Pittsburgh, Pennsylvania. *Received March 23, 1966; revised June 15, 1966.* Supported by the U. S. Public Health Service, National Institute of Arthritis and Metabolic Diseases, Grant AM-09083, awarded to N. H. S.

† Address after Sept 1966: Belfer Graduate School of Science, Yeshiva University, New York, N. Y.

[1] D-Lanthionine would also be eluted in this area.

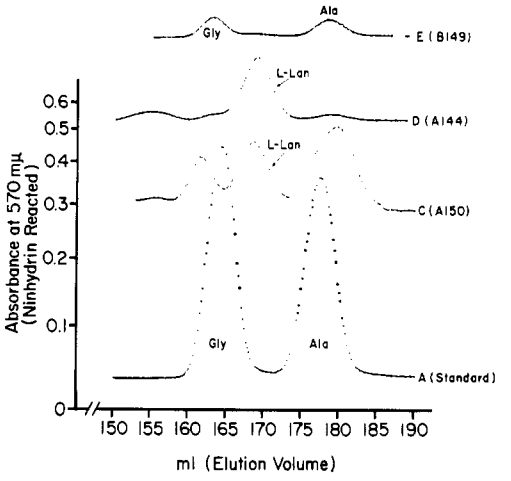

FIGURE 1: Technicon amino acid analyses of fractions prepared from acid-treated chick embryo. Curve A is the amino acid standard. Curve C shows amino acids present in the trichloroacetic acid protein precipitate (after extraction with hot trichloroacetic acid) after 6 N HCl hydrolysis. Curve D shows amino acids present in the free amino acid pool of the embryo. Curve E shows the amino acids present in the "collagen fraction" (hot trichloroacetic acid soluble fraction) after 6 N HCl hydrolysis. The preparation of the samples is described in the text.

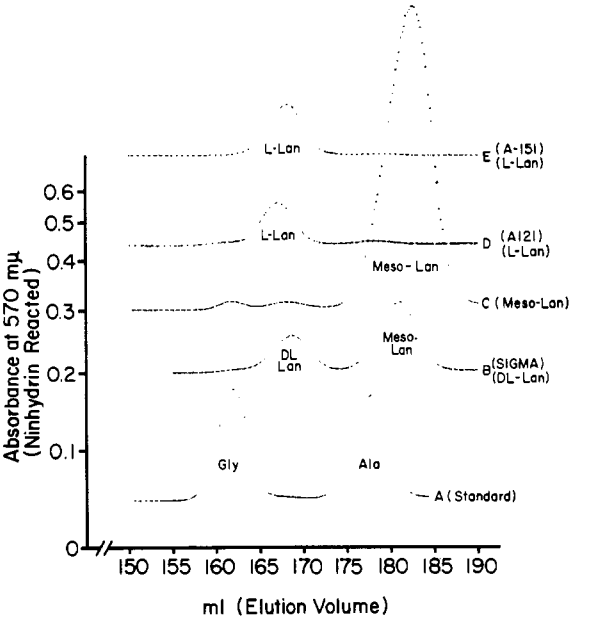

FIGURE 2: Technicon amino acid analyses of crystalline compounds prepared from the chick embryo. Curve A represents the amino acid standard. Curve B represents Sigma DL-lanthionine. Curve C represents crystalline *meso*-lanthionine isolated from the acid hydrolysate of the heat-treated chick embryo extract. Curve D represents the crystalline L-lanthionine isolated from the free amino acid pool of the acid-treated (nonheated) embryo extract. Curve E represents the crystalline L-lanthionine isolated from heat-treated embryo extracts after 6 N HCl hydrolysis.

Figure 2, curve D (A121); its infrared spectrum is displayed in Figure 3. This infrared spectrum is identical with that of authentic L-lanthionine[2] (Figure 4). These data prove that L-lanthionine does occur naturally in chick embryos. Each 15-day-old embryo contained approximately 30 μg of L-lanthionine.

After the completion of the work described here, Rao *et al.* (1966) reported that L-lanthionine is a naturally occurring amino acid in some insect larvae. Their infrared spectrum of L-lanthionine is nearly identical with that shown in this paper; however, their published sign of rotation for L-lanthionine is opposite from that found by us and other investigators[3] (Brown and du Vigneaud, 1941; Schoberl and Wagner, 1947).

It is tempting at this point to speculate that the difunctional molecule L-lanthionine may act, in certain proteins, as a cross-linking amino acid. The polyfunctional amino acids desmosine and isodesmosine (Thomas *et al.*, 1963) and the difunctional amino acid lysinonorleucine (Franzblau *et al.*, 1965) have been shown to be cross-linking amino acids in elastin. Finally, it may be significant that embryonic tissue has been the source for the isolation of naturally occurring L-lanthionine as reported both by us and by Rao *et al.* (1966).

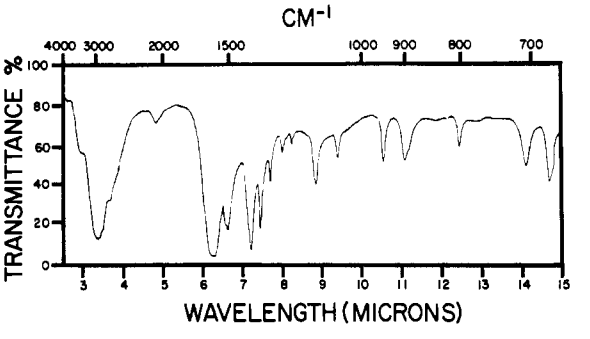

FIGURE 3: The infrared absorption spectrum (KBr) of L-lanthionine (A121) obtained from the free amino acid pool of acid-treated (nonheated) chick embryo. This spectrum was obtained on a Perkin-Elmer Infracord Model 137B spectrometer.

Evidence for the Presence of Bound L-*Lanthionine in Protein of Acid-Treated Embryo Extracts.* The hydrolysate of the trichloroacetic acid insoluble protein (after extraction with hot trichloroacetic acid) showed the presence of a ninhydrin-positive compound with the elution characteristics of L-lanthionine [Figure 1, curve

[2] Kindly supplied by Professor Vincent du Vigneaud, Department of Biochemistry, Cornell Medical College, New York, N. Y.

[3] The synthetic method of Schoberl and Wagner (1947) used by Rao *et al.* (1966) should have yielded the L isomer.

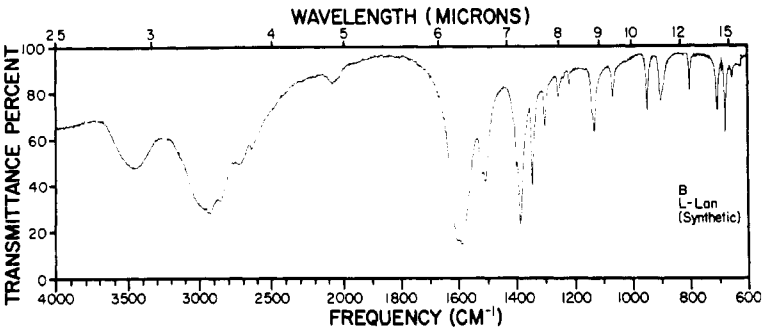

FIGURE 4: The infrared absorption spectrum (KBr) of synthetic L-lanthionine. This spectrum was obtained on a Perkin-Elmer Model 521 spectrometer.

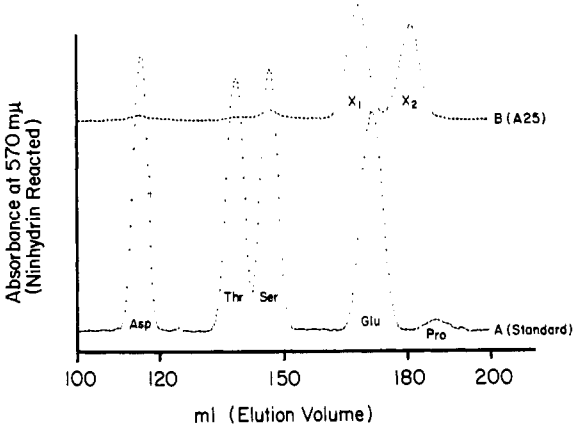

FIGURE 5: Spinco No. 120 amino acid analysis of the acid hydrolysate of heat-treated chick embryo. The standard amino acid elution pattern is shown in curve A. The preparation of the sample A125 (curve B) is described in the text.

C (A150)]. This finding strongly suggests that L-lanthionine is present in the bound form in chick embryo protein and not absorbed into the protein since the hot trichloroacetic acid extract, designated the "collagen fraction" (Peterkofsky and Udenfriend, 1963), is devoid of L-lanthionine as shown in Figure 1, curve E (B149).

Since the free amino acid pool of the acid-treated embryos showed only the L isomer, and 6 N HCl hydrolysis does not racemize the amino acid, it is unlikely that the ninhydrin-positive peak (Figure 1, curve C) that follows the L-lanthionine peak is *meso*-lanthionine. Furthermore, we have found that L-lanthionine was not found in fetuin,[4] albumin, or ribonuclease after the hydrolysates were treated with Dowex 50 resin and washed with the acid gradient and eluted before being subjected to amino acid analyses.

Isolation of L- *and Meso-Lanthionine from Heat-Treated Chick Embryo Extracts.* The Spinco No. 120 amino acid analysis (Figure 5) shows the amino acids obtained from Dowex 50 resin after the acid hydrolysate of the heat-treated chick embryo extract had been subjected to a dilute HCl gradient wash (Daughaday and Mariz, 1962) and 6 N HCl elution (see Experimental Section for details). It can be seen that the preliminary wash of the resin with dilute HCl eluted aspartic acid, threonine, serine, glutamic acid, and proline almost completely. However, it was noted that two ninhydrin-positive materials, designated X_1 and X_2, were present in the glutamic acid and proline elution areas. Thus, if the initial acid gradient wash had not been performed, it would not have been possible to demonstrate the presence of these two amino acids (X_1 and X_2 were subsequently identified as L-lanthionine and *meso*-lanthionine, respectively, and the data necessary for these identifications are presented below). When the single-column amino acid analysis system was used (Piez and Morris, 1960), these two amino acids were eluted in the glycine–alanine areas as shown in Figures 1 and 2.

The thorough study of the action of heat and dilute alkali (pH 8 and 10) on wool by Cuthbertson and Phillips (1945) showed that a mixture of lanthionine isomers (*meso* and DL) could be isolated from the hydrolyzed protein. Evidence presented by these workers strongly suggested that any lanthionine found was formed chemically from cystine. The rationale for this conclusion was that the disulfide bond of cystine was cleaved to give L-cysteine and α-aminoacrylic acid. These two acids then underwent a condensation to give lanthionine. The condensation predicts that a mixture of *meso*- and L-lanthionine (in equal amounts) should result if the reaction conditions are such as to preclude concomitant racemization of L-cysteine, L-cystine, and/or L-lanthionine. Since Cuthbertson and Phillips (1945) obtained *meso*- and DL-lanthionine, it seems that at least one of these amino acids did racemize under the conditions employed in their experiments. It is noted that both L- and *meso*-lanthionine were indeed present in the heat-treated embryo hydrolysate

[4] The fetuin was first heated at pH 7.2 (490 mg in 150 ml of 0.067 M phosphate buffer) at 121° for 3 hr before acid hydrolysis.

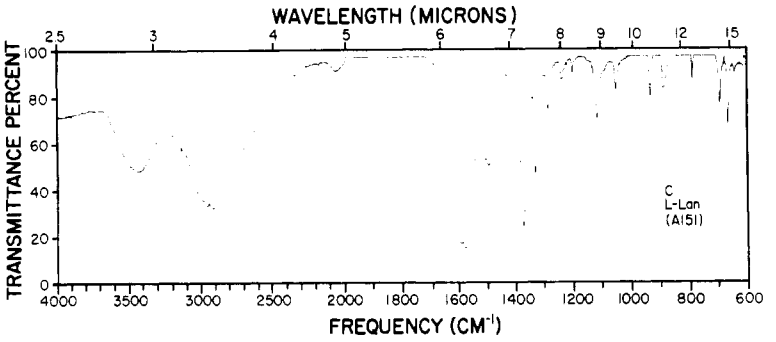

FIGURE 6: Infrared absorption spectrum (KBr) of L-lanthionine obtained from acid hydrolysis of heat-treated chick embryo extract. The spectrum was obtained on a Perkin-Elmer Model 521 spectrometer.

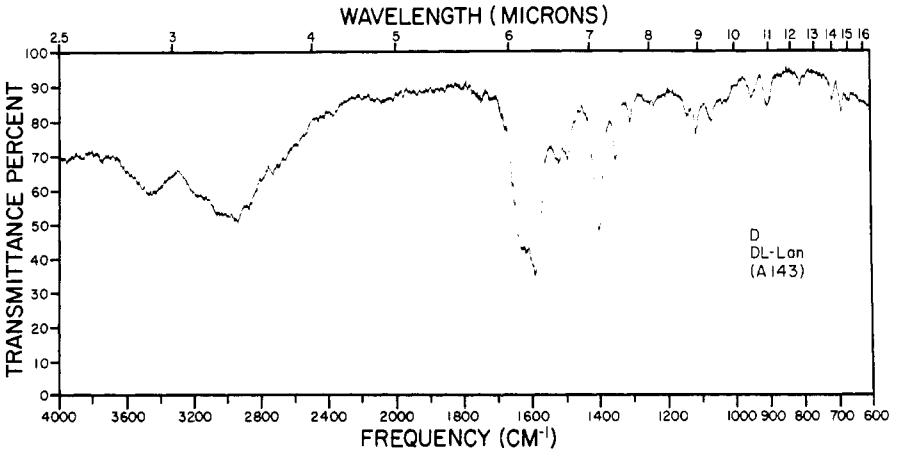

FIGURE 7: Infrared absorption spectrum (KBr) of DL-lanthionine obtained by alkali racemization of L-lanthionine. The spectrum was obtained on a Beckman IR9 spectrometer.

extract (Figure 5, curve B). To our knowledge, this is the first report of obtaining a mixture of lanthionines containing only *meso* and L isomers. Under the experimental conditions used here, no racemization of any of the amino acids involved had occurred.

We have found that L-lanthionine does racemize in 2.5 N NaOH solution at room temperature. It is assumed that milder basic conditions would also cause racemization. The material that was recovered after 7 days from the basic solution showed no optical rotation and its infrared absorption spectrum in KBr differed in several respects (at 1110 and 1530 cm^{-1}) from the L isomer as shown in Figures 6 and 7. It is well known that in solution, the infrared absorptions of a DL mixture and either the D or L isomer are identical unless the DL mixture is a racemic compound. Koegel *et al.* (1955) showed that the solid-state infrared absorption characteristics of optically active amino acids differed somewhat from their racemic mixtures. In addition, an amino acid analysis showed no *meso*-lanthionine to be present in the recovered crystalline material, which is in complete agreement with its infrared spectrum. The infrared spectrum of the *meso* isomer (Figure 8) is markedly different from either the L isomer or DL mixture. This racemization is of particular interest for it might be expected that a mixture of racemic and *meso* isomers should have been produced. Since no *meso*-lanthionine was found and the racemization had probably reached equilibrium, it is likely that the *meso* isomer, in basic solutions, is of higher energy than either the D or L isomer. Stabilization of the two isomers, D or L, could be brought about by intramolecular hydrogen bonding between the carboxylate anion and the amino group. This kind of spatial orientation is not possible in the *meso* isomer.

Proof of Identity of X_1 and X_2 as L- *and meso-Lanthionine Isomers.* The identification of X_1 as L-lanthionine was made from the following data. The melting point of X_1, 290–295° (darkens at 251°), agrees well with that reported by Brown and du Vigneaud (1941) for L-lanthionine (darkens, 245°; 293–295°). α-Amino nitrogen analyses[5] by the method of Van Slyke (1929)

[5] The analyses were kindly performed by Mr. A. Lo Monte in the laboratory of Dr. D. D. Van Slyke, Brookhaven National Laboratory, Upton, Long Island, N. Y.

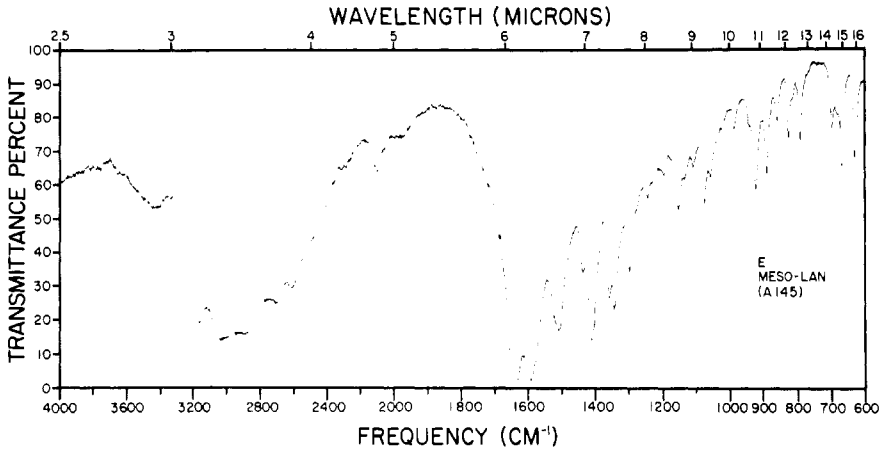

FIGURE 8: The infrared absorption spectrum (KBr) of *meso*-lantionine crystallized from the hydrolysate of the chick embryo. The spectrum was obtained on a Beckman IR9 spectrometer.

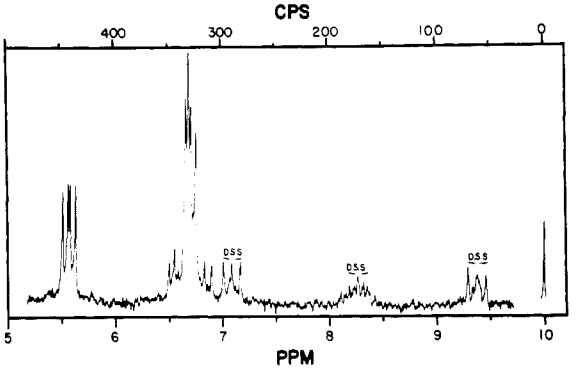

FIGURE 9: The nmr spectrum of D_2O–DCl solution of L-lanthionine isolated from the hydrolysate of heat-treated embryo extracts. The spectrum was obtained on a Varian HA-100 spectrometer with sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal reference standard.

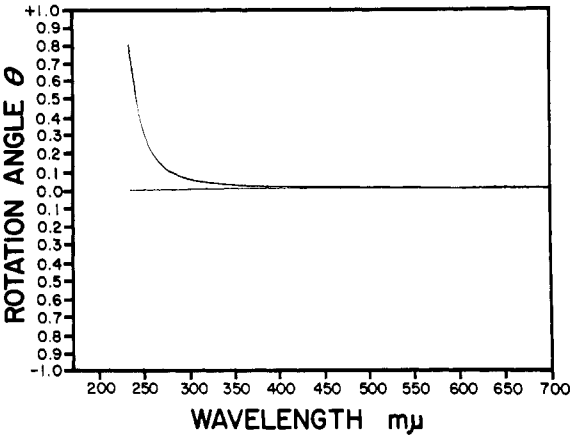

FIGURE 10: Optical rotatory dispersion curve of L-lanthionine (crystallized from the chick embryo after heat treatment and acid hydrolysis). The concentration was 4.3 mg in 0.5 ml of 2.5 N NaOH. The curve was obtained on a Model ORD/UV5 (Japan Spectroscopic Co., Ltd., Tokyo, Japan) spectrophotometer.

showed that X_1 contained two α-amino nitrogen atoms (*Anal.* Calcd for lanthionine: N, 13.29. Found: N, 13.68, 13.38). Two α-aminocarboxyl groups were shown to be present in X_1 by the amount of CO_2 obtained from reaction with ninhydrin[5] (Van Slyke *et al.*, 1941) (*Anal.* Calcd for lanthionine: C, 11.52. Found: C, 10.64). The nuclear magnetic resonance[6] spectrum of X_1 (Figure 9) is an ABX type. This spectrum was analyzed as an ABX case and the parameters thus obtained are $J_{AB} = 15.13 \pm 0.05$, $J_{AX} = 7.47 \pm 0.05$, $J_{BX} = 4.31 \pm 0.05$, and $\Delta\gamma_{AB} = 10.80 \pm 0.1$ cps.[7] These are in excellent agreement with those parameters reported by Glasel (1965) for L-cystine which is a good model compound for L-lanthionine. The mass spectrum of X_1 exhibited peaks at *m/e* 172, 145, 127, and no peak for a parent molecular ion at 208, corresponding to that expected for lanthionine. This result was initially misleading, but the mass spectrum of commercially available DL-lanthionine (Sigma Chemical Co.), which contains some of the *meso* isomer, was obtained and found

[6] Abbreviation used: nmr, nuclear magnetic resonance spectrum.

[7] The nmr spectrum was analyzed exactly as a three-spin problem, using the computer program LAOCOON II (Castellano and Bothner-By, 1964). We thank Dr. S. Castellano for his assistance with this analysis.

to be identical with that of X_1. Thus, it appears that lanthionine readily loses two molecules of water to give a fragment which would have a molecular ion, *m/e*, at 172.

Conclusive evidence that X_1 is either D- or L-lanthionine was obtained from a comparison of its infrared spectrum (Figures 3 and 6) to that of authentic synthetic L-lanthionine (Figure 4). It is readily seen that they are identical. In addition, it should be noted that the infrared spectrum of L-lanthionine differs from that of DL-lanthionine (particularly at 1110 and 1530 cm^{-1}) and differs markedly from that of meso-lanthionine (Figures 7 and 8). That the isolated amino acid X_1 is indeed L-lanthionine is shown by the optical rotatory dispersion curves of both alkaline and acidic solutions (Figures 10 and 11). The compound gives a positive rotation throughout the ultraviolet and visible ranges.

Brown and du Vigneaud (1941) reported that the specific rotation of L-lanthionine at the sodium D wavelength was +8.4° at 22° in 2.4 N NaOH. Schoberl and Wagner (1947) reported that the specific rotation of the L isomer was +19.9° at 11° in 1.0 N NaOH at the sodium D wavelength. The calculated specific rotations of the isolated L-lanthionine in acidic and alkaline solutions are shown in Table I.

TABLE I: The Specific Rotation of L-Lanthionine in Acidic and Alkaline Solution at 22° at Different Wave-Lengths in the Ultraviolet Region.[a]

λ (mμ)	$[\alpha]_D$ (deg) 1 N HCl	2.5 N NaOH
232	+4500 (max)	—
240	+2800	+1400
250	+1400	+800
280	+417	+233

[a] The concentration of L-lanthionine in 1 N HCl was 3.6 mg/0.5 ml; in 2.5 N NaOH the concentration was 4.3 mg/0.5 ml. The values were calculated from the optical rotation curves shown in Figures 10 and 11.

The proof of the identity of X_2 as *meso*-lanthionine is presented in Figure 8. The infrared absorption spectrum of this compound in the region between 900 and 1400 cm^{-1} is identical with the partial spectrum reported by Blackburn and Lee (1955).

Experimental Section

Isolation of L-Lanthionine from the Amino Acid Pool of Acid-Treated Chick Embryo Extract. Batches of 30 15-day-old chick embryos were homogenized with 200-ml volumes of cold H_2O for 45 sec in a Waring blender. The homogenate (equivalent to 144 embryos)

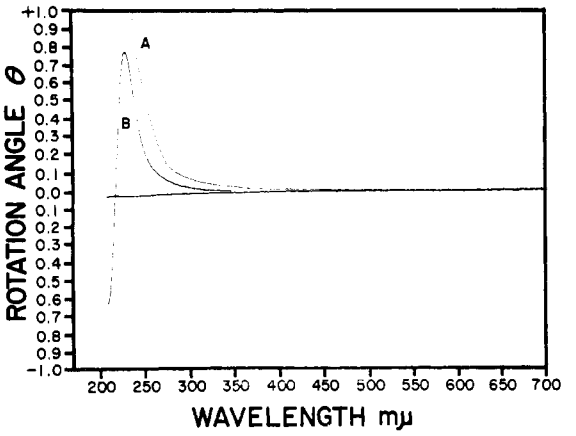

FIGURE 11: Optical rotatory dispersion curve of L-lanthionine (crystallized from the chick embryo after heat treatment and acid hydrolysis). The concentration in curve A was 3.6 mg in 0.5 ml of 1 N HCl; in curve B the concentration was 1.8 mg in 0.5 ml of 1 N HCl. The spectra were obtained with a Model ORD/UV5 (Japan Spectroscopic Co., Ltd., Tokyo, Japan) spectrophotometer.

was centrifuged in the cold and the insoluble material was washed with cold H_2O. The clear supernatant and washings (1425 ml at pH 6.9) were adjusted to pH 3.5–3.7 at 4° and allowed to settle overnight at 4°. The insoluble material was then removed by centrifugation. An equivalent of 66 embryos (650 ml) was applied to a 2 × 25 cm Dowex 50×8 (H^+) (AGW 200–400 mesh) column and the column was then washed with the dilute HCl gradient system described by Daughaday and Mariz (1962), utilizing 500 ml of 0.1 N HCl and 250 ml of 1.0 N HCl. The gradient-washed resin was eluted twice with 100-ml volumes of 6 N HCl (30 min/elution). The 6 N HCl eluates were combined and concentrated to dryness *in vacuo* at 70°. The residue was dissolved in 100 ml of H_2O and reconcentrated to dryness; this procedure was repeated three to five times. The residue was then dissolved in 8 ml of buffer (pH 2.2) (Moore *et al.*, 1958) containing 10% mannitol and applied to a 2 × 150 cm Spinco Type 150 A[8] resin (H^+ form) and eluted at 50° with citrate buffer (pH 3.24) (Moore *et al.*, 1958) at a rate of 120 ml/hr; 10-ml fractions were collected. The L-lanthionine was usually eluted after approximately 750 ml had passed through the column. The fractions containing L-lanthionine were located by ninhydrin determinations (Moore and Stein, 1954) on 0.2-ml aliquots of fractions 70–100. The L-lanthionine fractions were combined and applied to a 2 × 25 cm Dowex 50×8 (H^+ form) and washed free of buffer with 1500 ml of H_2O. The amino acid was eluted from the column with 1.0 N NH_4OH. The material was dried with a heat lamp and washed

[8] Spinco Type 150A is a sulfonated styrene–divinylbenzene copolymer resin 8% cross-linked.

several times with cold H_2O. The crystalline material (2.0 mg) was subjected to an amino acid analysis and it showed a single ninhydrin spot [Figure 2, curve D (A121)]. Evidence that this acid-treated extract did not contain *meso*-lanthionine is shown in Figure 1, curve D (A144). This curve (curve D, Figure 1) showed the presence of only the L isomer of lanthionine in the acid-treated embryo extracts that were first purified by cycling through Dowex 50×8 (H^+ form) utilizing the dilute HCl gradient as described above.

Evidence for the Presence of Bound L-*Lanthionine in Protein of Acid-Treated Embryo Extracts.* Chick embryo homogenate (100 ml, 15-day-old, equivalent to seven embryos) was clarified by centrifugation and adjusted to pH 3.5 at 4°; the clear supernatant obtained after centrifugation was adjusted to 10% trichloroacetic acid concentration. The protein precipitate (equivalent to 3.5 15-day-old chick embryos) was washed four times with cold 5% trichloroacetic acid solution. The pellet was then suspended in 25 ml of 5% trichloroacetic acid and heated on a steam cone (90°) for 60 min; after cooling to room temperature, the "collagen-free" precipitate (Peterkofsky and Udenfriend, 1963) was removed by centrifugation. The precipitate was suspended in 45 ml of 6 N HCl and hydrolyzed at 121° for 5 hr. The hydrolysate was concentrated to dryness *in vacuo* at 70°, dissolved in H_2O, and reconcentrated three to four times. The residue was dissolved in 25 ml of H_2O (pH 1.5) and applied to a 0.9 × 22 cm column of Dowex 50×8 (H^+) and washed with the HCl gradient system described above. The resin was then eluted with 6 N HCl and concentrated to dryness. The residue was dissolved in H_2O, applied to the 0.9 × 22 cm Dowex column, and washed with dilute acid gradient. The equivalent of one embryo (6 N HCl eluate of the acid-washed resin) was analyzed with the Technicon amino acid analyzer. The compound (L-lanthionine) is present in this hydrolysate [Figure 1, curve C (A150)].

To 25 ml of the "collagen fraction," 25 ml of 12 N HCl was added and the solution was hydrolyzed at 121° for 5 hr. The material was then cycled through the 0.9 × 22 cm Dowex 50×8 (H^+) resin as described above. The 6 N HCl eluate (equivalent to one embryo) was analyzed with the Technicon amino acid analyzer [Figure 1, curve E (B149)] and shown to be free of L-lanthionine.

Isolation of L-*Lanthionine and meso-Lanthionine from Hydrolyzed Heat-Treated Chick Embryo Extracts.* Batches of 30 15-day-old chick embryos were homogenized with 200-ml volumes of cold H_2O for 45 sec in a Waring blender. The homogenate (pH 6.75–6.90) was autoclaved at 121° for 3 hr; after cooling the material was strained through gauze and finally clarified by centrifugation. The supernatant was diluted with an equal volume of 12 N HCl and hydrolyzed at 121° for 5 hr (approximately 700-ml volumes were placed in 1-l. Pyrex bottles). Acid-washed, white rubber stoppers were inserted and secured with wire. The hydrolysate, after cooling, was concentrated to dryness *in vacuo* at 70°. The residue was dissolved in 100 ml of H_2O and reconcentrated. This procedure was repeated three to four times to remove excess acid. The equivalent of 20 embryos was dissolved in approximately 90 ml of H_2O; the solution (pH 1–2) was filtered and applied to a 0.9 × 22 cm column [Dowex 50×8 (H^+)] and washed with the 0.1–1.0 HCl gradient system as described above. The acid-washed resins of three Dowex 50 columns (equivalent to 60 embryos) were combined and eluted twice with 100-ml volumes of 6 N HCl; the 6 N HCl eluates were concentrated to dryness *in vacuo* at 70°. The concentration procedures were repeated three times after the addition of 50-ml volumes of H_2O to each dried residue. The dried residue was then dissolved in 5 ml of pH 2.2 buffer (Moore *et al.*, 1958) containing 10% mannitol. The mixture was filtered and applied to a 2 × 150 cm column of Spinco Type 150 A^8 resin (H^+ form) and eluted at 50° with citrate buffer (pH 3.24) (Moore *et al.*, 1958) as described above. The fractions containing L-lanthionine and *meso*-lanthionine were located by ninhydrin determinations (Moore and Stein, 1954) on 0.2-ml aliquots of fractions 70–100. The *meso* isomer (X_2) was eluted after the L isomer X_1 as shown in Figure 5. The fractions of each isomer were combined separately. The L-isomer fractions obtained from 288 embryos were applied to a 2 × 25 cm column of Dowex 50×8 (H^+) and washed free of buffer with 1500 ml of H_2O. The amino acid was eluted with 1 N NH_4OH. The eluate was dried under a heat lamp. The compound was recrystallized from small volumes of hot H_2O. The amino acid analysis of three-times-recrystallized material (33 mg from 576 embryos) showed a single peak that corresponded to L-lanthionine [Figure 2, curve E (A151)]. The crystalline *meso* isomer was obtained by the same procedures described above for the isolation and crystallization of the L isomer (Figure 2, curve C).

Racemization of L-*Lanthionine to* DL-*Lanthionine.* The D isomer was formed by racemization of the L compound; L-lanthionine was dissolved in 2.5 N NaOH (17 mg in 0.5 ml of alkali) and the solution was allowed to stand at room temperature for 7 days. After this period the solution was neutralized and applied to a 2 × 25 cm Dowex 50×8 (H^+) resin column. The column was washed with 1500 ml of H_2O to remove salt. The amino acid was eluted with 1 N NH_4OH and crystallized by the procedures described above.

Acknowledgment

One of us (N. H. S.) wishes to thank Dr. Lloyd M. Jackman (Department of Chemistry, University of Melbourne, Victoria, Australia) for helpful discussions. We wish to thank Mr. Ross Pitcher, Varian Associates, Pittsburgh, Pa., for the nmr spectrum.

References

Blackburn, S., and Lee, G. R. (1955), *Analyst 80*, 875.
Brown, G. B., and du Vigneaud, V. (1941), *J. Biol.*

Chem. 140, 767.
Castellano, S., and Bothner-By, A. A. (1964), *J. Chem. Phys. 41*, 3863.
Cuthbertson, W. R., and Phillips, H. (1945), *Biochem. J. 39*, 7.
Daughaday, W. H., and Mariz, I. K. (1962), *J. Lab. Clin. Med. 59*, 741.
du Vigneaud, V., and Brown, G. B. (1941), *J. Biol. Chem. 138*, 151.
du Vigneaud, V., Brown, G. B., and Bonsnes, R. W. (1941), *J. Biol. Chem. 141*, 707.
Franzblau, C., Sinex, F. M., Faris, B., and Lampidis, R. (1965), *Biochem. Biophys. Res. Commun. 21*, 575.
Glasel, J. A. (1965), *J. Am. Chem. Soc. 87*, 5472.
Horn, M. J., and Jones, D. B. (1941), *J. Biol. Chem. 139*, 473.
Horn, M. J., Jones, D. B., and Ringel, S. J. (1941), *J. Biol. Chem. 138*, 141.
Horn, M. J., Jones, D. B., and Ringel, S. J. (1942a), *J. Biol. Chem. 144*, 87.
Horn, M. J., Jones, D. B., and Ringel, S. J. (1942b), *J. Biol. Chem. 144*, 93.
Koegel, R. J., Greenstein, J. P., Winitz, M., Birnbaum, S. M., and McCallum, R. A. (1955), *J. Am. Chem. Soc. 77*, 5708.
Moore, S., Spackman, D., and Stein, W. (1958), *Anal. Chem. 30*, 1190.
Moore, S., and Stein, W. (1954), *J. Biol. Chem. 211*, 907.
Peterkofsky, B., and Udenfriend, S. (1963), *J. Biol. Chem. 238*, 3966.
Piez, K. A., and Morris, L., (1960), *Anal. Biochem. 1*, 187.
Rao, D. R., Ennor, A. H., and Thoyse, B. (1966), *Biochem. Biophys. Res. Commun. 22*, 163.
Schoberl, A., and Wagner, A. (1947), *Chem. Ber. 80*, 379.
Thomas, J., Eisden, D. F., and Partridge, S. M. (1963), *Nature 200*, 651.
Van Slyke, D. D. (1929), *J. Biol. Chem. 83*, 425.
Van Slyke, D. D., Dillon, R. T., MacFadyen, D. A., and Hamilton, D. (1941), *J. Biol. Chem. 141*, 627.