Incorporation of Orally Ingested Lysine-\(^{14}\)C into Egg Protein

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Hurwitz and Bornstein\(^1\) calculated the protein and amino acid requirements of laying hens using two models in one of which they assumed that total or some amino acids used for synthesizing egg albumen were derived from break down of tissue protein. They assumed that 2 g of tissue protein was necessary to synthesize 1 g of albumen, because the sulfur amino acid content of tissue was half that of egg albumen. But the protein ingested should be digested to amino acid and absorbed. The absorbed amino acids get into the blood and are mixed with those derived from the tissue where proteins are synthesized and degraded. Therefore, to promote effective amino acid utilization, it is necessary to elucidate the flow of dietary amino acids in laying hens. In this paper, the flow rate of orally ingested lysine-\(^{14}\)C into the excreta, respiratory carbon dioxide and eggs were studied.

Materials and methods

Eight Single Comb White Leghorn hens aged 60 weeks were supplied amino acid diet (Table 1) for 10 days. The food consumed and weight of egg were recorded at 3:00 P.M. every day. After a cluck for each hen was determined, the six hens were orally ingested with 25.0 \(\mu\)Ci of L-lysine-\(^{14}\)C (342 mCi/m mol, The Radiochemical Centre, Amersham) in a gelatin capsule after oviposition on the 10th day on the experimental diet. The hens that had consumed the labelled lysine were housed in metabolism cages made of acrylic resin and allowed free access to diet and water. Respiratory carbon dioxide was monitored in 100 ml of 2N sodium hydroxide solution at two hour intervals for 12 hours. Daily excreta were homogenized, 5 ml of N hydrochloric acid was added and made up to a volume of 500 ml. Ten ml of homogenate was centrifuged at 3,500 rpm for 10 mins. The precipitate was

<table>
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<th>Table 1. Composition of diet (g/kg)</th>
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<tr>
<td>Corn starch</td>
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<td>Amino acid mixture(^1)</td>
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<td>Cellulose powder</td>
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<td>Calcium carbonate</td>
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<td>Dicalcium phosphate</td>
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<td>Agar agar</td>
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<td>Lard and soybean oil (1:1)</td>
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<td>Vitamin and mineral mixture(^2)</td>
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1) 100 g amino acid mixture contained (g) Arg-HCl, 7.9; Lys-HCl, 5.9; His-HCl, 3.1; Leu, 9.1; Ile, 5.8; Val, 7.0; Met, 3.3; Cys, 2.8; Phe, 5.5; Tyr, 4.3; Trp, 1.8; Gly, 3.3; Pro, 4.1 and Glu, 31.8. All except Gly were L-forms.

2) Not less than the NRC requirements (1977)\(^2\).

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washed with five ml of 0.001N hydrochloric acid and centrifuged again. The mixed supernatant was made up to a volume of 20 ml and the precipitate was dissolved with five ml of 2N sodium chloride solution.

The eggs laid after isotope ingestion were divided into egg white, yolk, shell, yolk membrane and shell membrane. To egg shells 50 ml of 40\% trichloroacetic acid (TCA) was added. Carbon dioxide liberated from the egg shell was absorbed with 10 ml of 2N sodium hydroxide. After the shell melted, TCA soluble fraction and insoluble fractions were separated by centrifugation at 3,500 rpm for 10 mins. The precipitate was washed with five ml of 0.001N hydrochloric acid and centrifuged again. The mixed supernatant (shell TCA soluble) was made up to a volume of 20 ml and the precipitate (shell membrane) was dissolved with five ml of 2N sodium chloride solution.

The egg white of the second and yolk of the fifth egg which incorporated most radioactivity were homogenized and fractionated on Sephadex G-25 (Pharmacia Fine Chemicals) using 0.002M glycine phosphate buffer solution, pH 7.0 separately.

The obtained protein fractions were fractionated on DEAE cellulose, using 0.002M glycine phosphate buffer solutions, containing increasing concentrations of sodium chloride as described by Perkinson\(^3\). The radioactivities of all preparations were determined using a nonion-toluene scintillator (4 g of PPO in one litter of nonion-toluene mixture 3:7) by a liquid scintillation counter (Aloca LSC 900). The nitrogen content of excreta were determined by a microkjeldahl method and that of egg fractions was determined on a spectrophotometer (Shimadzu QV-50) at 280 nm.

Results and discussion

All hens consumed almost equal amounts of diet and maintained constant body weight throughout the experiment. However, egg production was lower than that observed on a corn-soybean meal stock diet which contained 0.71\% lysine during the seven days before the experiment.

The data of three hens out of six, which maintained high egg production after lysine-\(^14\)C ingestion are presented here.

Fig. 1 shows the recovery of \(^14\)C in egg fractions. The highest \(^14\)C activity was found in the second egg white. The low activity in egg white of the first egg shows that the synthesis of protein had been finished before the isotope had reached the site of synthesis. On the other hand, the highest \(^14\)C activities of shell CO\(_2\) and shell TCA soluble fractions of the first egg suggested that the isotope had reached the synthesis site before their formation. The specific activity per mg protein changed along with the pattern of the \% recovery of \(^14\)C in each fraction examined.

In spite of the fact that plasma free lysine-\(^14\)C concentration decreased to a low level within a few hours after ingestion, the highest radioactivity was found in the second egg white and the appearance of \(^14\)C at a relatively high level in the third and fourth egg white. These observation agreed well with the results of Salter et al.\(^4\) injected intravenously hydrolysate of U-\(^14\)C-protein from chlorella and of Hassell et al.\(^5\) injected lysine-\(^14\)C. They suggested that the labeled albumen synthesized initially when the plasma \(^14\)C was at its highest level was not all deposited in the first active egg, and that a proportion remained
Fig. 1. Time course of total activities (left) and specific activities (right) in egg white (A), yolk (B), yolk membrane (C), shell membrane (D), shell CO₂ (E) and shell TCA soluble fractions (F) for 10 days after ingestion of L-lysine-U-¹⁴C

and was deposited together with freshly synthesized albumen in subsequent eggs. The low levels of ¹⁴C found in later eggs were presumably derived from amino acids released into the pool through the turnover of labeled tissue proteins. However, it is not clear that ¹⁴C found in eggs derived from digestive tract directly or indirectly via tissues.

The recovery of ¹⁴C in egg yolk increased till the fifth egg and then decreased gradually. This observation is consist with our knowledge of ovulation physiology⁹. It has been shown that the ova protein are synthesized in the liver and deposited in ovary over a period of eight days and that the maximum growth increment occurs around five days before ovulation. Before isotope ingestion, the first egg yolk protein had been completely synthesized. Thus, ova from the second to the fifth day incorporated a higher proportion of lysine-¹⁴C than on the first day. The maturing ova after the second day continued to deposit lysine-¹⁴C derived from the inflowing pool through the turnover of labeled tissue proteins and reached a maximum by the fifth egg.

Table 2 shows the distribution of radioisotope after ingestion. Since the expired ¹⁴C in
Table 2. Distribution of isotope in egg, excreta, respiratory carbon dioxide (12 hours) and body of laying hens 10 days after ingestion of L-lysine-U-\(^{14}\)C (\%)

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<thead>
<tr>
<th></th>
<th>egg</th>
<th>excreta</th>
<th>respiratory CO(_2)</th>
<th>retained (calculated)</th>
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<tr>
<td>Egg</td>
<td>41.2±1.8</td>
<td>3.0±0.1</td>
<td>2.8±0.2</td>
<td>53.0±1.6</td>
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Fig. 2. Absorbance and DPM of fractions of second egg white after ingestion of lysine-\(^{14}\)C on Sephadex and DEAE cellulose

Fig. 3. Absorbance and DPM of fractions of fifth egg yolk after ingestion of lysine-\(^{14}\)C on Sephadex and DEAE cellulose
CO₂ decreased sharply within 12 hours. The ¹⁴C in CO₂ for the following 10 days was not more than 50% of that observed during the first 12 hours. Assuming that when the retained isotope in hen's body (53.0%) is released again, the ratio of isotope distributed into excreta and respiratory carbon dioxide will be less than 10% of the retained, thus the utilization of dietary lysine may be higher than 90% in the hens fed the diet containing 0.70% lysine.

The fraction patterns and the total activity on Sephadex G-25 and DEAE cellulose of egg white and yolk are shown in Figs. 2 and 3. Only one fraction was obtained from each Sephadex pattern, but more than seven fractions were taken from the DEAE cellulose pattern for egg white of the second egg. The specific radioactivity as expressed DPM (decay per min) per absorbance, ranged in small width, e.g. 3,070, 3,170, 2,850, 2,650 and 3,350 for peak I to V in Fig. 2.

In the case of yolk (Fig. 3), three fractions were taken from a Sephadex G-25 pattern, and more than 10 fractions were taken from the DEAE cellulose pattern for the main fraction. The specific radioactivity (DPM/absorbance) was also the same in each fraction e.g. 7,400, 7,800, 8,600, 7,300 and 7,400 for the peak from I to V. These results suggested that each protein was synthesized at the same time from the same pool, since the plasma free lysine⁻¹⁴C injected decreased so rapidly7).

**Summary**

1. The laying hens aged 60 weeks were fed amino acid diet with 0.70% of lysine. On the 10th day, they were ingested 25.0 μCi of L-lysine-U-¹⁴C just after oviposition.
2. The recoveries % of dose for 10 days after ingestion in excreta, respiratory carbon dioxide (12 hours) and eggs were 3.0, 2.8 and 41.2%, respectively.
3. The highest ¹⁴C activities in egg white, yolk membrane and shell membrane were found in the second egg, those in shell CO₂ and shell TCA soluble fractions were found in the first egg and those in the yolk were found in the fifth egg.
4. The same specific activity in each fraction of the second egg white and of the third yolk by Sephadex G-25 and DEAE cellulose column suggested that each protein in egg white and yolk might be synthesized at the same time from the same pool.

**Acknowledgement**

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**Literatures**

経口投与したリジン-¹⁴Cの卵蛋白質へのとりこみ

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1. 60週令の産卵鶏にリジンを0.70％含有する飼料を給与し、10日目の放卵直後にL-リジン-U-¹⁴C 25.0μCiを経口的に投与した。
2. 投与後10日間の排泄物、呼気炭酸ガス（12時間）、卵からの¹⁴Cの回収率は3.0、2.8、41.2％であった。
3. 最も高い¹⁴C活性が得られたのは、卵白、卵黄膜、卵殻膜では第2卵、卵殻中のCO₂血分、TCA可溶血分では第1卵、卵黄では第5卵であった。
4. 第2卵の卵白、第3卵の卵黄をセファデックスG-25、DEAEセルロースカラムで分画したときの各画分の比放射能が等しかった。このことは各画分は同じところで同時に合成されている可能性を示唆した。

（家禽会誌, 20, 231～236, 1983）