Egypt. J. Anim. Prod. 21, No. 2, pp. 211—220 (1981)

The Distribution of some Amino Acids in Hen's Egg During Incubation

R.S. Farag, A.M. Youssef and H. Salem

Biochemistry Department, Faculty of Agriculture, Cairo University, Egypt.

A STUDY of the distribution of 17 amino acids has been carried out on the white and yolk during incubation of the hen's egg using one-dimensional paper chromatography. A modified paper chromatographic method was described to separate 10 amino acids as well defined spots without overlapping in a reasonable time. Between the 3rd and 17th day of incubation most of the amino acids approximately accumulated in the embryo at the same rate. For the first 10 days of incubation, the yolk was the sole source of amino acids for the embryo. Whilst from the 10th until the 17th day the white supplied the major portion of amino acids needed by the embryo.

The bird's egg contains most of the varied materials of life in amounts that are sufficient to ensure the growth and development of the embryo. For instance, Davidorich and Fermandez (1960) mentioned that the whites and yolks of eggs contained all of the essential amino acids. Also, Marchetti (1952) and Evans *et al.* (1945) found the same results.

Several investigators have studied the change in the amounts of some amino acids during incubation of the hen eggs. For instance, Cedrangolo *et al.* (1953) have studied the relation between proline, hydroxyproline, arginine and glutamic acids during incubation. Also, Grau (1947) noted that the percentage of the amino acids in the yolk protein remains unchanged during the development. On the other hand, Calvery (1932) found that the tyrosine content of the embryo protein decreased with age while that in the yolk and white remained constant during egg incubation. The results of Abderhalden and Kempe (1967) indicated that the amounts of glutamic and glycine remained constant throughout the incubation period. An interesting study was done by Rupe and Farmer (1955) on the distribution of 14 amino acids on the white, yolk and embryo during incubation of the hen eggs.

One can conclude that variation in the amounts of some amino acids in the egg as the chick embryo develops are occurred. However, there are disagreements concerning the nature of these changes. Because of the discrepancies among the previous investigators and the limited number of amino acids studied, the present study by means of paper chromatographic methods has been undertaken to review the distribution of 17 amino acids in the whites and yolks of hen's egg during incubation.

Material and Methods

Materials

Fayoumi eggs obtained from the Poultry Farm of the Faculty of Agriculture, Cairo University were used throughout this study. Eggs were collected from hens kept on a diet of constant composition for 3 weeks before the beginning of collection. Only fertilized Fayoumi eggs were incubated at 38° with a relative humidity of 60 to 70%. Twenty eggs were removed from the incubator daily until the 17*th* day of incubation for assay purposes and the eggs in which the embryos ceased its development were excluded.

Preparation of egg-whites and yolks for analysis

The residual egg whites were separated from the yolks, embryos, fluids, chalaza and other integuments. The separation was accomplished by making a small hole in the shell and shell membranes and the egg whites were dried under vacuum in a desiccator containing phosphorous pentoxide. In case of residual egg yolks, the eggs were heated on a boiling water bath for 10 min and the yolks were carefully separated from any venules and other constituents. The egg yolks were dried in the same way as in egg whites. Both egg whites and yolks were ground in a mortar and stored in the refrigerator until analysis.

Methods

1. *Hydrolysis of the egg-whites and yolks*

Samples ranging from 0.4 to 0.8 g were hydrolyzed with 6 N HCl as described by Atfield and Morris (1960). Tryptophan was hydrolyzed by 14% Ba $(OH)_2$ as mentioned by Baker and Khan (1957).

2. *Separation of amino acids*

Three different solvents were employed for the separation of 17 amino acids using Whatman No. 1 as follows :

a. The method of Baker and Khan (1957) was applied for the separation of aspartic, glutamic, serine, glycine, threonine and alanine using phenol buffered at pH 12 (solvent 1) as mobile phase for 24 hr at 20°. The papers were immersed on the buffer solution (pH 12) and allowed to air dry before the application of the hydrolysate solution.

b. An improved method used by the authors for the separation of 10 amino acids namely : cystine, lysine, histidine, arginine, tyrosine, methionine, valine, phenylalanine and leucine-isoleucine as a pair. The first 4 amino acids were clean separated using the mobile phase of n-butanol-acetic acid-water (solvent 2) in the ratio 4:1:5 (v/v) in 48 hr at 20°. The remainder amino acids were well fractionated using n-butanol-acetic acid-water (solvent 3) in the ratio 4:1:1 (v/v) in 44 hr at 15°. The filter papers were dipped in the solvent 2 and 3 and allowed to air dry before the application of the hydrolysate solutions.

3. *Procedure*

The quantitative determination of the 17 amino acids using three different solvent systems by descending one-dimensional paper chromatography technique was applied. Each hydrolysate was spotted separately on the paper alongside the standard amino acid mixtures (2, 4, 6 and 8 uM). The chromatograms were placed in the chromatographic chamber outside the troughs overnight to saturate the chromatograms with the chromatographic atmosphere and allowed to develop. Once the chromagatograms had run to the desired stage, they removed from the chromatographic chamber and rapidly driving off the solvents by dipping them in an ether solution.

4. *Colour formation and estimation of amino acids*

The cadmium ninhydrin reagent was used to locate the amino acids as mentioned by Heathcote and Haworth (1969). The optical

densities of the clear red solutions were measured at 500 nm using 1 cm glass cells in the Pye Unicam spectrophotometer model Sp 600. Tryptophan was determined colourimetrically as described by Opienska-Blauth *et al.* (1963).

Results and Discussion

One of the most direct methods for studying the amino acids metabolism during the incubation period was to follow up the changes in amino acid content of egg whites and yolks during incubation period and compare it with the unincubated eggs at the start of the experiment. This method was applied in the present work.

The distribution of the 17 amino acids in the incubated hen's egg has been studied using one-dimensional paper chromatographic technique. A number of attempts have been done to develop a new and simple paper chromatographic procedure for the isolation and quantitative determination of amino acids in a reasonable time. These endeavours had led to throw a light on some factors which affect to a great extent the resolution of the amino acids. For instance, the time required to equilibrate the solvent mixture before separating the two layers of immiscible solvents and to equilibrate the chromatograms with the chromatographic atmosphere before putting them in the mobile phase and also the developing temperature as well were among the critical factors which affect the amino acids separation. Excellent resolution was achieved if the conditions described in the method section were followed.

The values for the various amino acids are expressed as percentage, in the egg whites and yolks. No values are given for the embryo and fluids. The amounts of the following amino acids of the unincubated egg whites were approximately the same as in egg yolks, e.g., aspartic (11% and 13%), glutamic (13.5% and 13%), serine (8.5% and 9.2%), glycine (4.5% and 4%), threonine (5% and 7%), alanine (6% and 7%), tryptophan (2.5% and 1.3%), tyrosine (3% and 3.5%), valine (5.9% and 6%), leucines (12% and 14%), and phenylalanine (6% and 5%). On the other hand, cystine (1.3% and 0.5%), lysine (6% and 3.5%), histidine (3% and 2%), arginine (7% and 3.5%) and methionine (3% and 1.8%) were found in whites approximately twice as much as in yolks.

The fact that the weight of the chick embryo increased exponentially as mentioned by Murray (1926) suggests that the increase of an amino acid in the embryo would also follow as exponential curve. By plotting the values of the various amino acids against time, one obtains in most cases a straight line (Figs. 2 and 3) showing that the accumulation of an amino acid follow the same pattern.

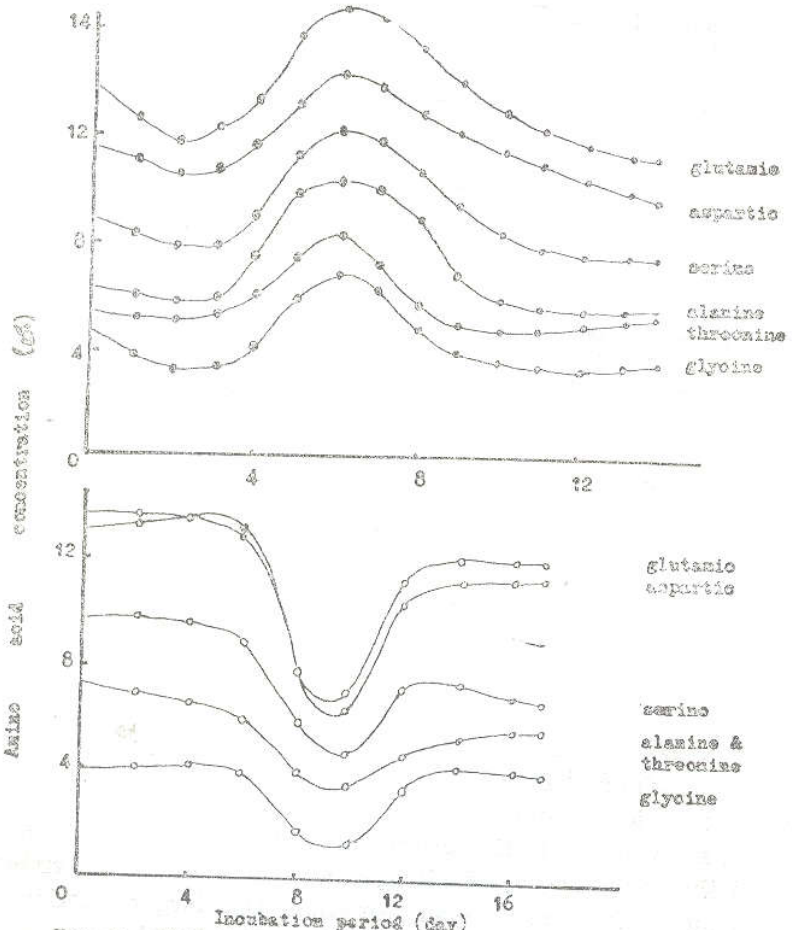

Fig. 1. Distribution of some amino acids in white (-●-) and yolk (-○-) hen's egg during incubation.

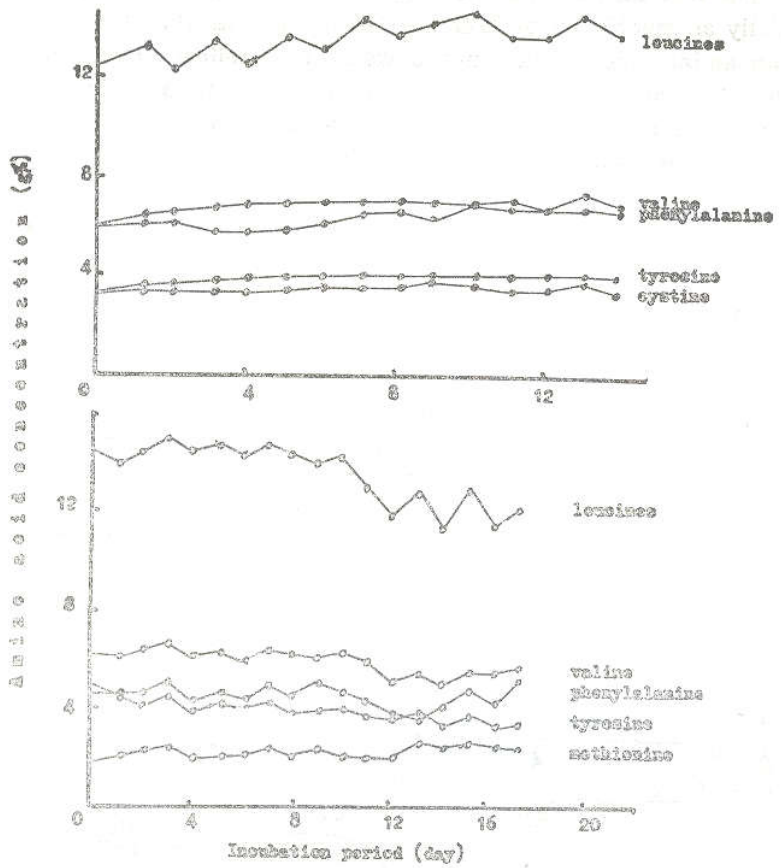

Fig. 2. Distribution of some amino acids in white (-o-) and yolk (-o-) hen's egg during incubation.

It is common knowledge that the egg losses solids during incubation. Fiske and Boyden (1926) stated that not more than 6% of the organic material oxidized was protein and 96% the egg protein is converted to tissue protein. Also, Needham (1931) mentioned that only 11 mg of nitrogen are excreted by the embryo development leads one to suspect that the larger portion of catabolized material is non-protein in nature and that only a small fraction of the total weight loss is due to destruction of amino acids.

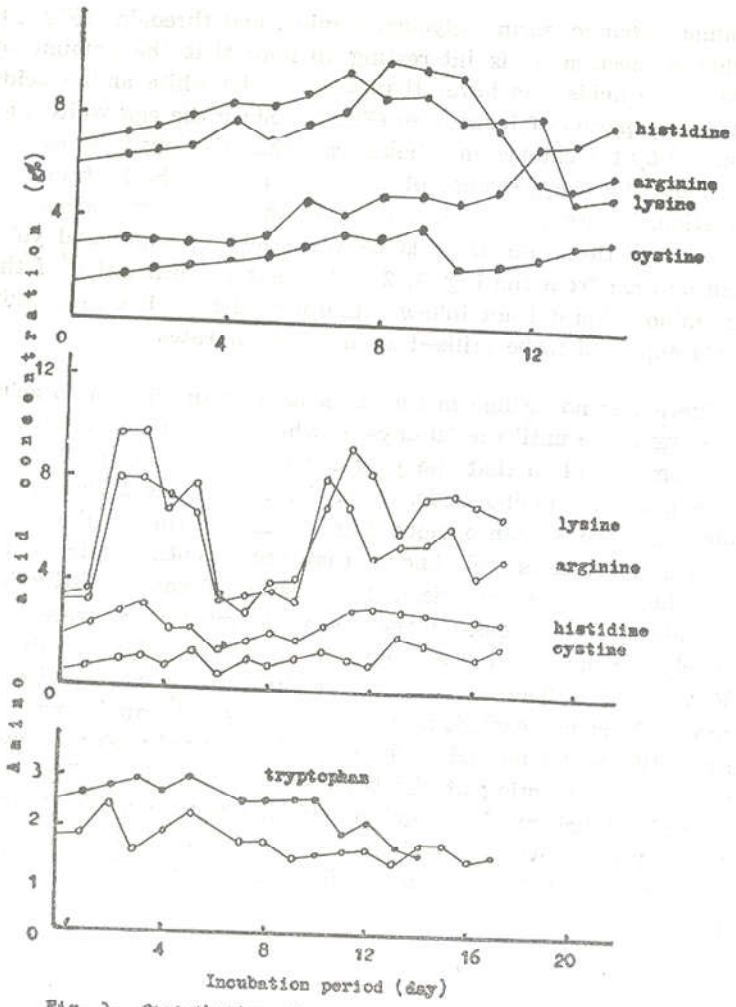

Fig. 3. Distribution of some amino acids in white (-●-) and yolk (-○-) hen's egg during incubation.

Rupe and Farmer (1955) mentioned that during the first 17 days of incubation, 43% of the yolk substances is utilized by the embryo. The results of the present work showed that the yolk furnished most of the material needed for the first 12 days of development as evidenced by the early decline in the amino acids :

glutamic, aspartic, serine, glycine, alanine, and threonine (Fig. 1). In this connection, it is interesting to note that the amount of these amino acids was lower than that of the white amino acids in the same period of incubation (Fig. 1). Since the egg white was consumed by the embryo in a faster rate than the egg yolk, as evidenced by the disappearance of the egg white in the 15*th* day of incubation. Therefore, one would expect that there was a sort of migration of these amino acids between the egg white and yolk. It can be seen from the Fig. 1, 2 and 3 that the utilization of the yolk amino acids did not follow a uniform pattern, but these substances appeared to be utilized at independent rates.

There was no decline in the concentration of the amino acids in the egg white until the 10 days at which the embryonic growth has become rapid so that the yolk can no longer adequately supply the necessary amino acids. From the 10*th* to the 17*th* days of incubation there was an obvious decrease in the white amino acids (Fig. 1 and 2). It is well known that the essential amino acids are an indispensable materials. The results of this investigation have indicated that some of the essential amino acids were continuously consumed and at a constant rate by the embryo as shown in Fig. 2 and 3. The possibility that the embryo can synthesize amino acids is not excluded, since there was only an increase in the quantity of lysine and arginine in the egg yolk (Fig. 3) over the above that existing at the beginning of incubation. The possibility of catabolism of the amino acids appears more likely since there is an evidence that arginine, lysine, leucine and tryptophan in egg white (Fig. 3) decrease during the end period of incubation.

References

Atfield, G.N. and Morris, C.J.O.R. (1960) The separation and quantitative determination of amino acids by high-voltage paper electrophoresis. **Biochem. J. 74, 37.**

Baker, B.E. and Khan, N.A. (1957) Determination of amino acids in undesalted hydrolysates by buffer filter paper chromatography. **J. Sci. Food and Agric. 8, 217.**

Calvery, H.O. (1932) Some chemical investigation of embryonic metabolism. **J. Biol. Chem. 95, 297.**

Cedrangolo, F., Fidanza, F., Quagliaxiello, E. and Gioia, A. (1953) Relation between proline, hydroxyproline, arginine and glutamic acid in the chicken egg during its embryonal development. **Arch. Sci. Biol. 37, 260.**

Davidovich, N.P. and Fernandez, M.V. (1960) Microbiological determination of amino acids in egg and milk. Anales. **Fac. Quim. Farm. Univ. Chile 12, 75.**

Evans, R.J., Butts, H.A., Davidson, J.A. and Bandemer, S.L. (1949) The amino acid content of fresh and stored shell eggs. **Poultry Sci. 28, 691.**

Fiske, C.H. and Boyden, E.A. (1926) Nitrogen metabolism in the chick embryo. **J. Biol. Chem. 70, 535.**

Grau, C.R. (1947) The phenylalanine and tyrosine contents of chicks and eggs. **J. Biol. Chem. 170, 661.**

Heathcote, J.G. and Haworth, C. (1969) An improved technique for the analysis of amino acids and related compounds on thin layers of cellulose. **J. Chromatogr. 43, 84.**

Marchetti, E. (1949) The lipids contained in kidney, testes and liver. Rass. Clin. Terap. E. Sci. Affini 48, 1.

Murray, H.A. (1926) Physiology ontogeny. A chicken embryo. XII. The metabolism as a function of age. **J. Gen. Physiol. 10, 337.**

Needham, J. (1931) «Chemical Embryology», Cambridge.

Opienska-Blauth, J., Charezinski, M. and Berbec. H. (1963) A new rapid method of determining trytophan. **Anal. Biochem. 6, 69.**

Rupe, C.O. and Farmer, C.J. (1955) Amino acid studies in the transformation of proteins of the hen's egg to tissue proteins during incubation. **J. Biol. Chem. 213, 899.**

دراسة كيميائية على الاحماض الامينية فى بياض وصفـــار البيض فى الدجاج الفيومى

رضوان صدقى فرج ، عبد المنعم يوسف وحسن سالم

كلية الزراعة ـ جامعة القاهرة

استخدم التحليل الكروماتوجرافى الورقى فى اتجاه واحد لفصل وتقدير الاحماض الامينية كميا فى بياض وصفار البيض خلال فترة التحضين وهى : جلوتاميك ـ امبارتيك ـ سيرين ـ الانين ـ تريونين ـ جليسين ـ ليوسين ـ ايزولسين ـ فالين ـ فينايل الانين ـ ثيروزين ـ مستين ـ منيونين ـ هستدين ـ ارجنين ـ ليسين ـ تربتوفان .

واستحدثت طريقة للتحليل الكروماتوجرافى الورقى امكن بواسطتها فصل عشرة اخماس امينية بصورة واضحة فى وقت مناسب حيث استخدم نظامين من المذيبات وهما : البيوتانول العادى ـ حامض الخليك ـ الماء بنسب ٤ : ١ : ٥ (حجم / حجم) لفصل السستين ـ ليسين ـ هستدين ـ ارجنين ـ ونفس مخلوط المذيبات السابقة بنسب ٤ : ١ : ١ (حجم / حجم) لفصل التيروزين ـ مثيونين ـ فالين ـ فينايل الانين ـ ليوسين ـ ايزولوسين . والجديد فى هذه الطريقة عن الطرق الاخرى هو نقع الورق الكروماتوجرافى فى المذيبات السابقة وتركها لتجف هوائيا قبل اجراء عملية التنفيط واجراء الفصل الكروماتوجرافى على درجـــة حرارة منخفضة ١٥ م .

واوضحت الدراسة انه خلال الفترة ما بين اليوم الثالث الى السابع عشر للتحضين يمتص الجنين الاحماض الامينية الاساسية بمعدل ثابت . ودلت نتائج التقدير الكمى للاحماض الامينية جلوتاميك ـ اسبارتيك ـ سيرين ـ جليسين ـ الانين ـ ثربونين أنه حتى اليوم الثانى عشر للتحضين على انخفاض كميتها فى الصفار بينما يقابله ارتفاع فى كميتها فى البياض مما يدل على حدوث انتقال هذه الاحماض الامينية من الصفار الى البياض خلال فترة نمو الجنين واستنتج ايضا احتمال تكوين أحماض امينية بواسطة الجنين النامى نظرا لارتفاع كمية الاحماض ليسين ـ ارجنين فى صفار البيض عن كميتها فى بداية التحضين ـ ولم يستبعد احتمـــال هدم بعض الاحماض الامينية مثل : الارجنين ـ ليسين ـ ليوسين ـ ايزوليوسين ـ تربتوفان نظرا لانخفاض كميتها فى بياض البيض عند نهاية فترة التحضين بالمقارنة بكميتها عند بداية التحضين