U.A.R. J. Anim. Prod., Vol. **11**, No. 1, pp. 19-40 (1971)

ELECTROPHORETIC STUDIES OF EGG WHITE PROTEINS

By

*H.M. Salem, A.M. Youssef and A.R. El-Harras

Paper electrophoresis technique was used for the analysis of egg white proteins of different birds belonging to different phylogenetic orders. Three hen varieties *i.e.* Rhode Island, Baladv and Fayoumi showed 9 protein bands. Different species such as Turkey, Sudani-duck, Pekin dvck and goose showed only 8 protein bands.

The soluble proteins of the Rhode Island egg white was subjected to extensive analysis. Different fractions were obtained when different concentrations of amm. sulphate were used in separarion. Ovalbumins showed 4 protein components, conalbumin was represented by one bend while ovomucoid contained 5 bands overlapped with those of ovalbumin. Globulin was separated to 3 fractions G_1, G_2 and G_3. A new fraction was separated at 60% amm. sulphate satu- ration and was fractionated into 3 bands.

For a long time egg proteins were consideerd important constituents of the human diet. This should be true in imperative since the egg must provide all the essential nutrients for the emberyonic development of the chick to the stage when oral nutrition becomes possible .

Bain and Deutsch (1948), presented the electrophoretic diagram couvering the examination of egg white proteins of 13 species of birds belonging to 6 different phylogenetic orders. A characteristic pattern was obtained for each species. It was possible to differentiate each species by this technique.

Lewis *et al.* (1948), studied the electropforetic pattern of egg white. Their pattern indicated 7 distinguishable components, ovalbumin A_1 and A_2, ovamucoid, conalbumin and three globulin G_1, G_2 and G_3. The identity of these components was established by comparsion with the pattern obtained from the purified components with the exception of the globulins.

The object of this investigation was the study of the protein components of eggs of different species in U.A.R. and an attempt of separation and identification of these proteins was performed.

*Biochemistry Division, Animal Production Department, Faculty of Agriculture, Cairo University, Giza.

Materials and Methods

In this study hen eggs of different varieties were used. These included the Balady, Fayoumi and Rhode Island. Eggs of different species belonging to different phylogenetic orders such as, Turkey, Sudani duck, Pekin Duck and Goose were also used. Egg samples were provided by the Higher Agricultural Institute Poultry Farm at Kafr-el-Sheikh.

Preparation of egg white proteins for paper electrophoresis :

Egg whites were separated from the egg yolk and chalazae, then homogenized by stirring. Pyridine-glacial acetic acid buffer solution was prepared (8 ml. pyridine, 2.5 ml. gl. acetic acid per litre of distilled water), then adjusted to PH 5.5. Egg white was diluted with the buffer solution in the ratio of 1:3 V/V and the mixture was centrifuged at 3500 r.p.m. The precipitate containing the insoluble impurities was discarded, while the supernatant solution containing the soluble proteins was subjected to paper electrophoresis.

Paper electrophoresis technique :

Methods described by Durrum (1950) were used utilizing pyridine acetic acid buffer (pH 5.5), Whattmann No. 1 filter paper strips and lasting for 16 hours under 220 volt.

After complete separation the strips were removed and the protein bands were localized after staining with azo-carmen B dye.

Quantitative determination of the protein bands :

The localized protein bands were estimated by a model MGE Berlin densitometer. The colour density of each pattern was recorded on a paper strip in the shape of a curve. The curve was divided into areas under each peak. Each area relative to the total area was measured by means of a planimenter. From these measurments the relative percentage of each protein was determined as shown in figs. : 1, 3, 5, 7, 9, 11, 13 and 15.

Fractionation Methods :

Different methods were applied for the separation of the different protein constitutions of the Rhode Island egg whites which were localized in the electrophoretic pattern.

These methods were as follows :

1.—*Precipitation with Ammonium sulphate :*

An aliquot (50 ml.) of the egg white solution in the pyridine / acetic buffer pH 5.5 was cooled to 1°C. A cooled saturated amfonium sulphate solution was gradually added until a turbid was occurred. The mixture was

then left in the cold for an hour until a precipitate was formed. The mixture was centrifuged, the precipitated proteins were redissolved, diluted to 50ml. with the buffer, then dialyzed against the same buffer and subjected to paper electrophoresis. The supernatant was filtered off and retained for further fractionation of the other protein components by the addition of saturated amm. sulphate solution in the same manner. Fractionation proceeded until no precipitate could be obtained.

2.—*Heat fractionation technique :*

The buffered egg white solution was heated to 100°C. on a water bath for 4 hrs. The mixture was centrifuged and the precipitate was discarded. The supernatant after filtration was subjected to paper electrophoresis.

3.— *Cold acetone or cold alcohol precipitation :*

This technique was applied for the separation of different protein constituents of ovomucoid fraction. The buffered protein solution (50 ml.) was cooled to 1°C., then cold acetone or ethanol was added dropwise. The precipitate when formed was centrifuged, redissolved, diluted to 50 ml. with the pyridine buffer and subjected to electrophoresis. The supernatant was filtered of and retained for fractionation of the other protein components using further addions of cold acetone or alcohol in the same manner until no precipitate could be obtained.

4.— *Dialysis techique :*

This method was applied for the separation of egg white globulins. Whole egg white was dialyzed against distilled water. A precipitate was formed. The mixture was centrifuged and the precipitate was dissolved in pyridine acetic buffer (pH 4.0). The solution was filtered, then subjected to paper electrophoresis.

5.—*Column chromatography fractionation technique* :

This method was used for the separation of the components of ovalbumin and ovomucoid. An ionic exchange column of carboxymethyl cellulose (CMC) was used. Two starting phosphate buffers with different concentration were used. The first was $NaHPO_4$ + NaH_2PO_4 0.01 M at PH 6.0.

The higher concentration was 0.1 M at the same pH.

Results

Fractions of Egg white Proteins :

1.—*Ovalbumin* :

The fraction obatined at 52% amm. sulphate saturation was electrophoretically similar to the ovalbumin fraction which was prepared according to the method of Srensen and Hyrup (1950). This fraction indicated the

presence of four bands in the electrophoretic analysis. They were represented as the bands 1, 2, 3, and 4, in the original egg wite pattern fig. 15. These four bands were separated from each other by using column chromatography technique as shown in figs. 16 to 21.

2.—*Conalbumin :*

The fraction obtained at 57%amm. sulphate saturation was electrophoretically represented by the band number 8 fig. 22. This fraction was found to be similar to conalbumin which was prepared by alcohol fractionation technique according to Bain and Deutsch (1948).

3.—*Unknown fraction.*

A fraction indicating electrophoretically the presence of three bands represented as 5,6, and 7 on the original electrophoretic pattern was obtained at 60% amm. sulphate saturation fig. 23.

4.—*Ovomucoid :*

The fraction obtained at 65%am. sulphate saturation was found electrophoretically similar to the non-coagulable protein known as ovomucoid which was prepared according to Hesselvik (1938), by heat coagulation. The electrophoretic analysis of this fraction revealed the presence of five bands as shown in fig. 24.

The first four bands of ovomucoid were also obtained as a fraction at 70% ethyl alcohol concentration, while the fifth band was obtained by 80% acetone concentration fig. 25. CMC. Column chromatography was used successfully to fractionate the three bands : 2, 3 and 4 from the 70% alcohol fraction of ovomucoid in a pure form as shown in figs. 26-29.

5.—*Globulins :*

This fraction was found to contain three proteins, and was elctrophoretically identified as that fraction known as globulins G_1, G_2 andG_3 which was also separated according to longworth *et al* (1940) and Alderton *et al.* (1945).

The first G_1 was obtained when 15-20% ethanol concentration was used as represented by band 9 in the original electrophoretic pattern fig. 30.

The second globulin G_2 was obtained at 37 % amm. sulphate saturation fig.32, while G_3 was obtained at 47 % amm. sulphate saturation. The two globulins G_1 and G_3 were also obtained wken the whole egg white was dialyzed against water and the formed precipitate was dissolved in pyridine / acetic buffer at PH 4.0.

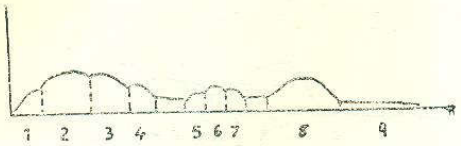

FIG. 1 Rhode Island

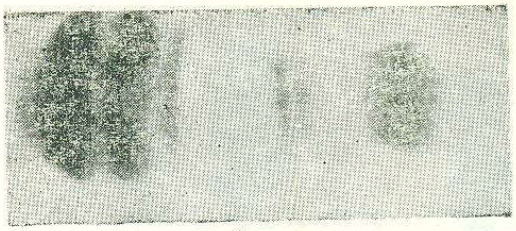

FIG. 2

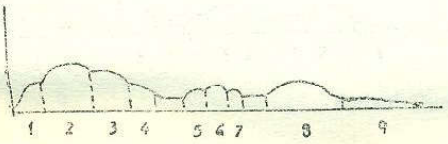

FIG. 3 Fayoumi

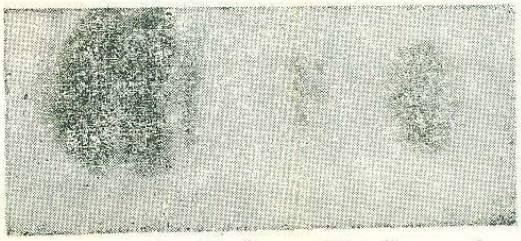

Fig. 4

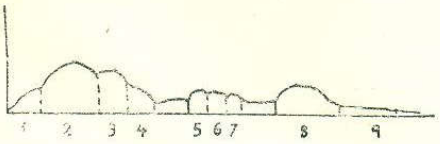

FIG. 5 Balady

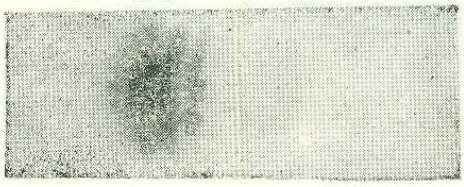

Fig. 6_Sudani duck Liverin

Electrophoretic curves and patterns of egg white proteins
(Pyridine - acetic buffer pH 5.5)

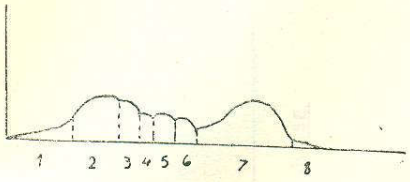

FIG. 7 Turkey

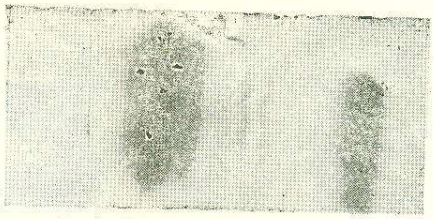

Fig. 8

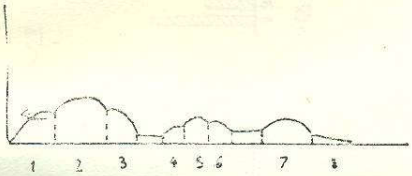

FIG. 9 Sudani Duck

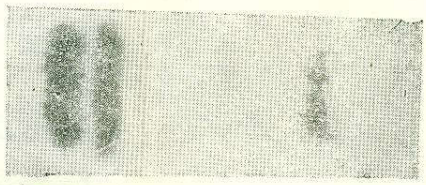

Fig.10

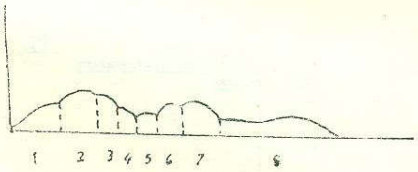

FIG. 11 Pekini Duck

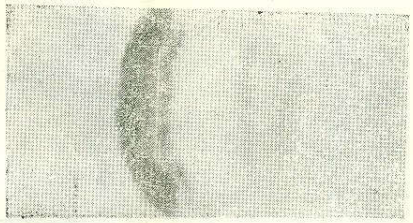

Fig.12

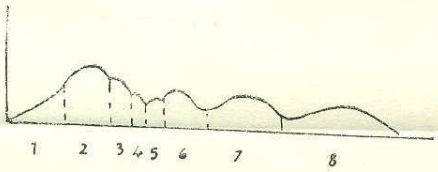

FIG. 13 Goose

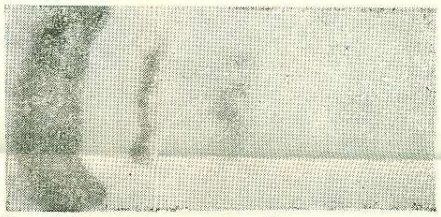

Fig.14

Electrophoric patterns 8 curves of egg white of different birds

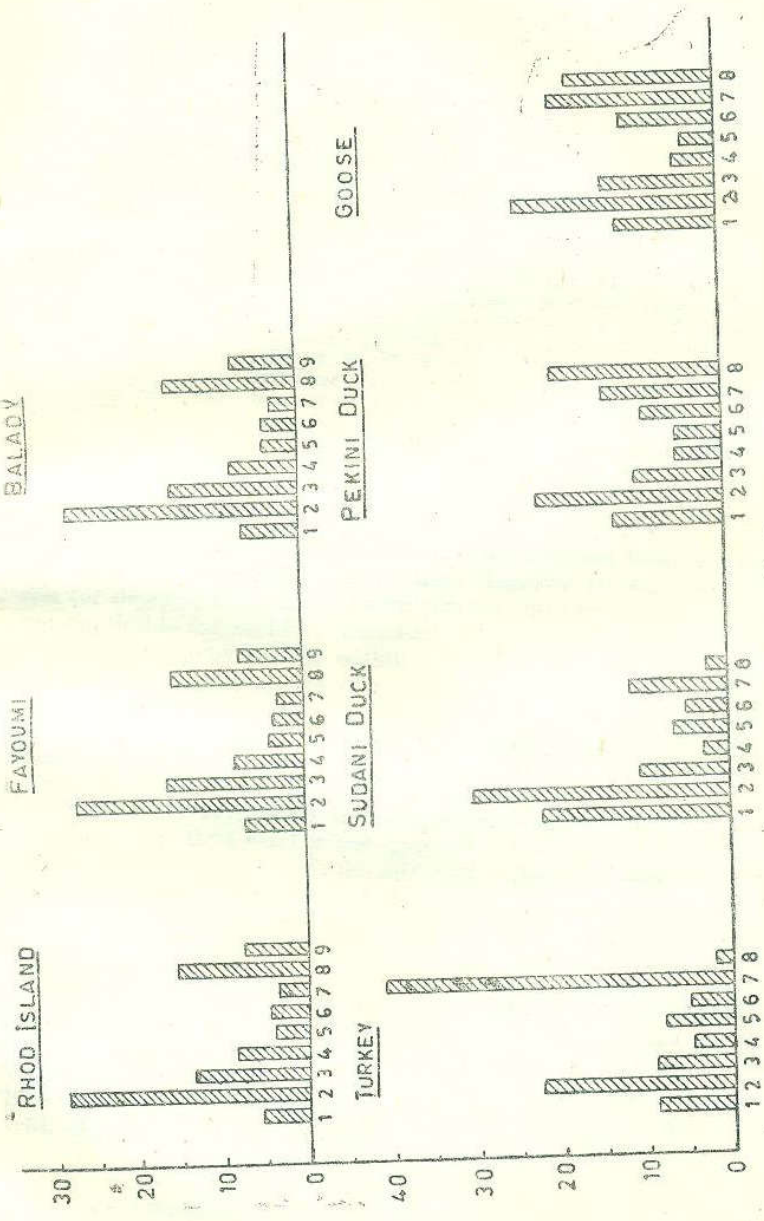

Fig 15.- Percentage of Protein in Different Birds

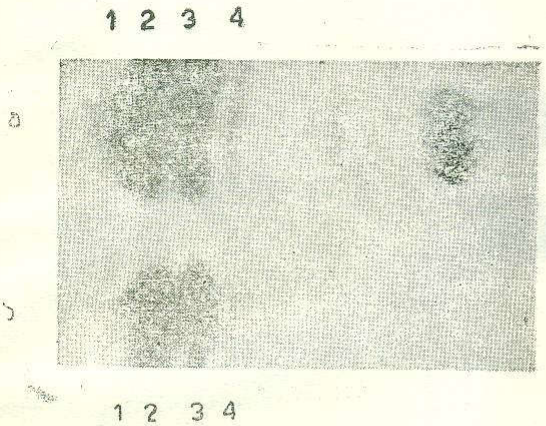

FIG. 16—Electrophoretic pattern of :
(*a*) Hen egg white (*b*) Ovalbumin fractions.
(Separated at 52% Amm. Sulphate Saturation)

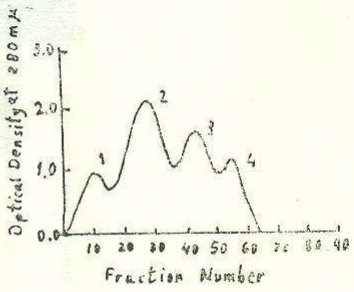

FIG. 17—Ovalbumin curve

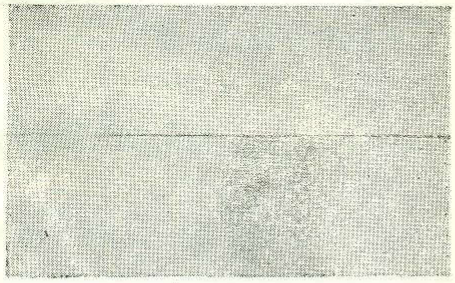

FIG. 18—Band 1

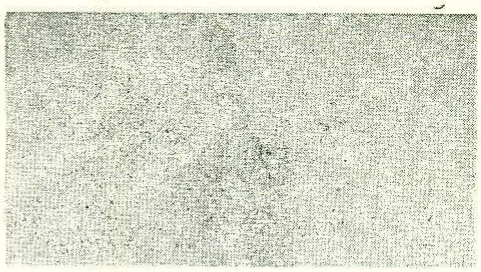

FIG. 19—Band 2

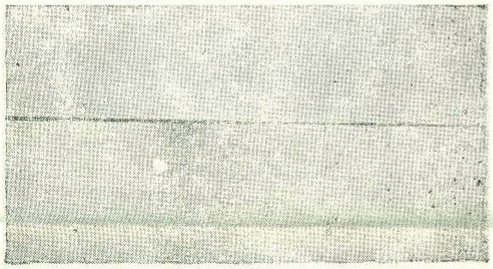

FIG. 20 —Band 3

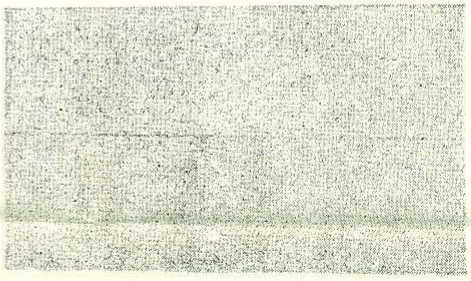

FIG. 21 —Band 4

Electrophoretic patterns of ovalbumin fractions.

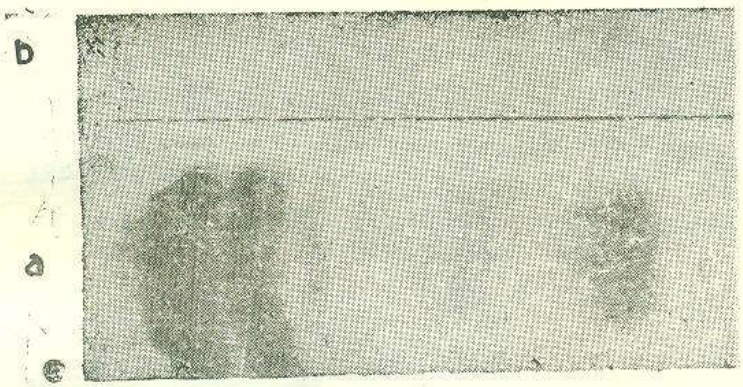

FIG. 22—Electrophoretic pattern of :

(*a*) Hen egg white

(*b*) Conalbumin fraction (Separated at 57% amm. Sulphate saturation).

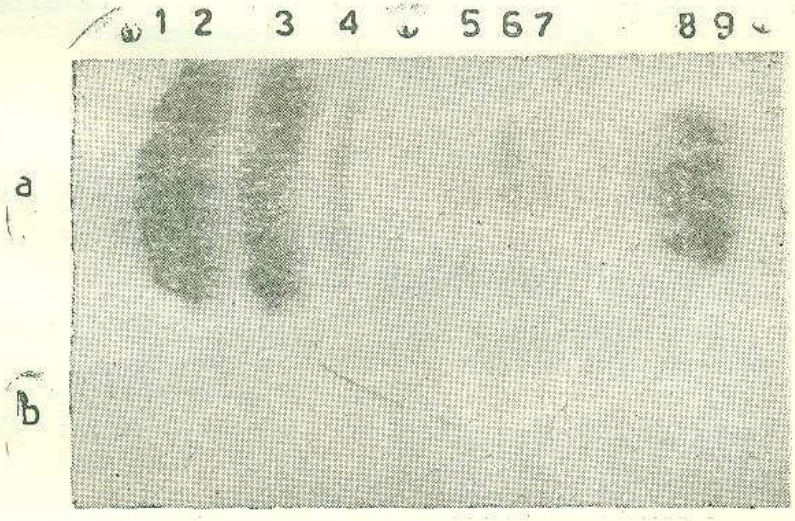

FIG. 23—Electrophoretic pattean of :

(*a*) Hen egg white

(*b*) Unknown fraction (5, 6, 7) separated at 60 amm. Sulphate saturation.

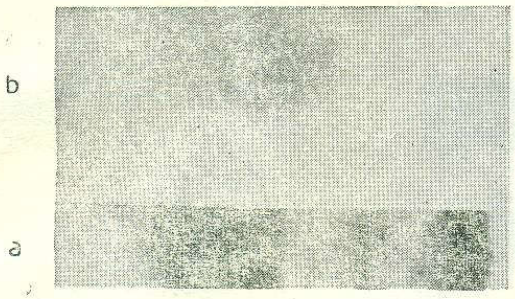

FIG. 24 —Electrophoretic pattern of :

(*a*) Hen egg white

(*b*) Non-coagulable ovomucoid fraction (65% amm. sulphate).

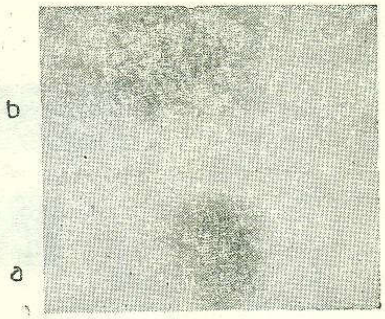

FIG. 25 —Ovomucoid Fractions :

(*a*) Separated at 80% acetone Co.

(*b*) Separated at 70% ethanol Co.

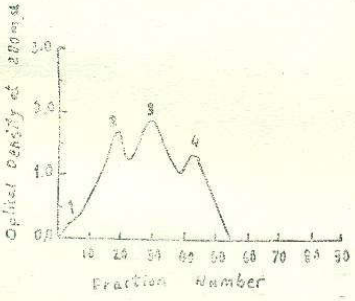

FIG. 26 —Ovomucoid Chromatograne Curve.

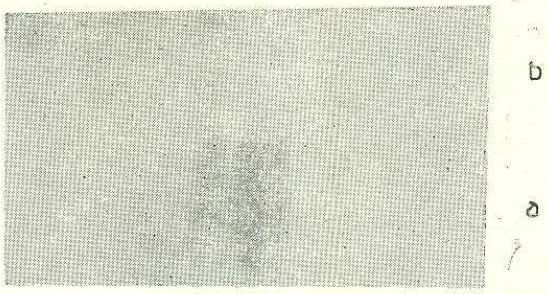

FIG. 27 —Ovomucoid Band 1

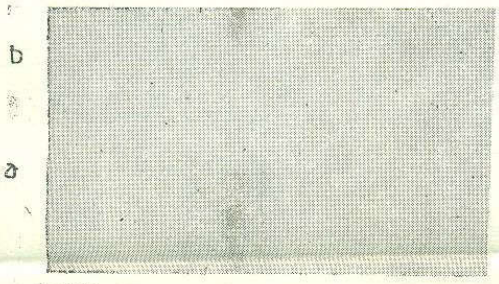

FIG. 28 —Ovomucoid Band 2

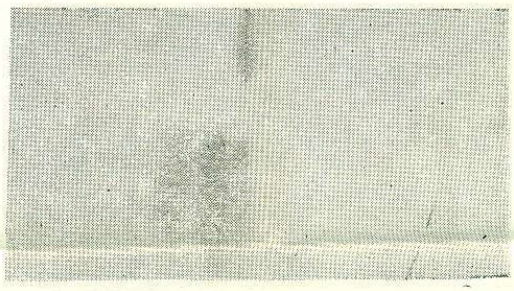

FIG. 29—Ovomucoid Band 3

Electrophoretic Patterns of ; (*a*) Ovomucoid fractions. (*b*) Ovomucoid bands 1, 2 & 3.

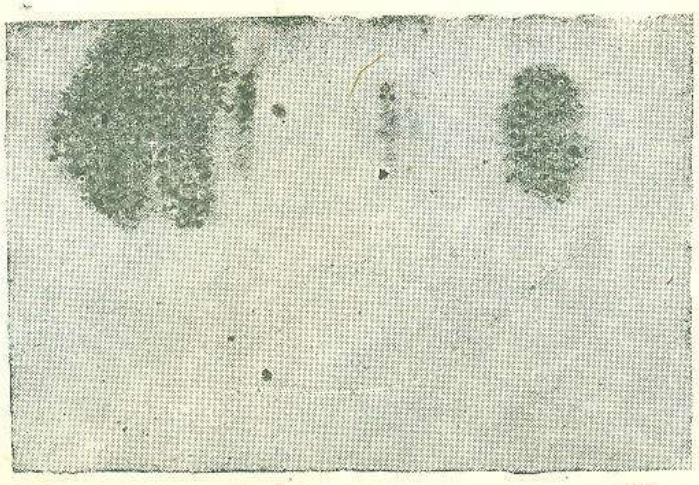

FIG. 30—Globulin Fractions

(*b*) G 1 (20% ethanol concentration)

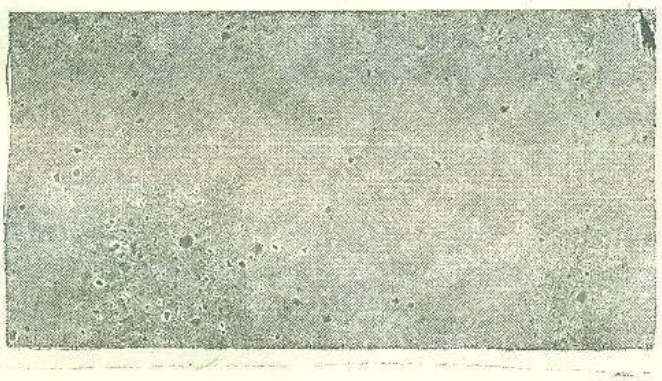

FIG. 31—Globulin Fractions

(*b*) G 2 (38% amm. sulphate saturation)

Electrophoretic Patterns of :

(*a*) Hen egg white.

(*b*) Globulin fractions (G1, G2).

Discussion

Although much work has been done by different workers on the egg protein constituents and their chemical composition, no definite results were reported concerning their electrophoretic studies.

This work compromised an examination by paper electrophoresis of the soluble proteins of egg white. The most suitable results were obtained when a pyridine/acetic acid buffer solution at pH 5.5 was used.

The most striking fact emerged from this work was the considerable differences in the electrophoretic patterns and diagrams of either different varieties of hen or different phylogenetic orders. These differences were not merely of degree, since some quantitatively important components of the proteins found in eggs were present in some species, but completely absent from others figs. 1—15. Bain and Deutsch (1947), reported the same results.

The amout of each protein differs from one electrophoretic pattern to another according to the density of the dye on the paper. The densitometer was used to measure the color of the bands located on the electrophoretic pattern.

The electrophoretic patterns of the whole egg white of the three hen varieties *i.e.* Rhode Island, Fayoumi and Baladi were identical in mobility having 9 distinct bands but slightly differed in the quantity.

On the other hand, the patterns obtained from Turkey, Sudani duck, Pekini duck and Goose showed only eight distinct bands with remarkable difference in quantity owing to their different phylogenetic orders figs. 1—15.

Different methods were used to separate each protein band located in the electrophoretic pattern of the Rhode Island variety in rather a pure state.

It was planned to separate the soluble proteins of the egg white using closer ranges of amm. sulphate saturations.

The fraction obtained at 52% amm. sulphate saturation was identified as ovalbumin and was found to contain four protein bands 1—4 fig. 2. Separation of the four components were achieved by using the ion exchange column chromatography, then followed by the electrophoretic analysis figs. 17—21. These results disagreed with those reported by Lewis *et al.* (1948), who postulated the presence of only two components. Other workers (5), (12) reported the presence of three components. These differences in results might be due to th different buffers used or to the different techniques applied.

The fraction represented as band 8 fig. 22 was identified as conalbumin. It might be concluded that conalbumin could be obtained at 57% amm. sulphate saturation. This result agreed with those obtained by Clark *et al.* (1963) and Hellhammer *et al.* (1958).

The fraction obtained at 65% amm. sulphate saturation was identified as the non-coagulable ovomucoid protein. This fraction when subjected to column chromatography then followed by electrophoretic analysis was found to contain five components. Ovalbumin and Ovomucoid were overlaped by each other since the five bands of ovomucoid were found on the same place where the ovalbumin usually migrates on the paper fig. 24—29. This may be due to the fact that in an electric field a protein moves at a rate determined by the size and shape of the molecule and by the number and kind of ionizied groups. Fractions that appear homogeneous by criterion of solubility may contain components that differ in rate of electrophoretic travel.

These results differed from those obtained by earlier investigators (6, 10, 12) who indicated electrophoretically the presence of one or two components.

A fraction obtained at 60% amm. sulphate saturation was separated electrophoretically into three bands fig. 23. These bands 5, 6 and 7 were not identified by any other investigators.

The fractions obtained by 20% ethanuol concentration or at 38% and 47% amm. sulphate saturation were identified as the globulins G_1, G_2. and G_3. G_1 showed the highest mobility of all egg white proteins representing band 9. The other two G_2 and G_3 were ovrlaped by the preeceding bands 7 and 8. These bands were previously identified by Longsworth *et al.* (1940).

REFERENCES

ALDERTON, G., WARD, W.H., AND FEVOLD, H.L.—Isolation of lysozoyme from egg white. J. *Biol. Chem.* **157**, 43 (1945).

BAIN, J.A., AND DEUTSCH, H.F.—An electrophoretic study on the egg white proteins of various birds. *J. Biol. Chem.*, **171**, 531–41 (1974).

——— .—Separation and characterization of conalbumin. *Ibid*, **172**, 247 (1948).

CLAK, J.R., OSUGA, D.T., AND FENNY, R.E.—Composition of avian egg white conalbumins., *J. Biol. Chem.*, **238**, 11, 3621 (1963).

DURRUM, E.L.—A micro-electrophoretic and microionophoretic technique. *J. Am. Chem. Soc.*, **72**, 2943–48 (1950).

EVANS, R.J.,AND BANDEMER, S.L.—Separation of egg white proteirs by paper electrophoresis. *J. Agr. Food Chem.*, 4, 80210 (1956).

FORSYTHE, R.H., AND FOSTER, 9.F.—Electropohoretic studies on whole egg white. *J. Biol. Chem.*, **184**, 377–83 (1950).

HELLHAMMER, D., AND HOGL, O.—Paper electrophoresis of egg proteins. MITT GEBEITE LEBENSON. *U. Hyg.*, **49**, 165–71 (1958); **50**, 84–110 (1959). (c.f *from Chem. Abs.*, **53**, 494, 20337 1959).

HESSELVIK, L.—*Z. Physiol. Chem.*, **254**, 144 1938); c.f *from Advances in protein Chem.* Vol. VI, 188. 1950).

LEWIS. 9. LONGSWORTH, R., CANNON, K., AND MACINNES, D.A.—An electrophoretic study of the proteins of egg white. *J. Am. Chem. Soc.*, **70**, 2580–89 1948).

LONGSWORTH, L.G., CANNON, K., AND MACINNES, D.A.; —An electrophoeretic study of the proteins of egg white. *J. An. Chem. Soc.*, **62**, 705–11 1940).

SORENSON, S.P., AND HOYRUP, H.—*Compt. rend. trav. lab. Carlesberg*, **12**, 167. c.f *from Advances in protein Chemistry vol.* VI. **188** (1950).

SUGANO, H.—Electrophoretic studies of chick egg white and process of its fractionation by sodium sulphate. *Seiutsu Butsuri Kayaku.* **1**, 41–45 1952). c.f. *from Chem. Abs.*, **51** 8836 (1957).

SUGANO, H.—Electrophoretic studies on proteins. Hen egg white. *J. Fac. Sci. Nugata Univ. Ser.*, **1**, **2**, 31–39. c.f *from Chem. Abs.*, **49**, 14069 (1955).

دراسة عن بروتينات بياض البيض بطريقة التقريد الكهربى

* حسن محمد سالم ، عبد النعم يوسف ، أحمد الهراس

الملخص

أجريت هذه الدراسة على بياض بيض مأخوذ من أنواع مختلفة من الطيور مثل الدجاج (بلدى ، فيومى ، رود ايلاند) والرومى ، والبط (سودانى ، بكينى) والأوز ، وذلك لمعرفة أنواع البروتينات المكونة لهذا البياض ، ووجد أن بياض الدجاج يحتوى على تسعة مكونات من البروتينات تتماثل مع بعضها فى الأصناف الثلاثة فى قدرتها على الهجرة على ورق الترشيح ولكن تختلف فيما بينها اختلافا بسيطا من حيث نسبتها المئوية .

أما بالنسبة لأنواع الطيور الأخرى فقد وجد أن بياض بيضها يحتوى على ثمانية مكونات من البروتينات فقط وتختلف عن بعضها فى قدرتها على الهجرة على ورق الترشيح وكذلك فى نسبتها المئوية .

وقد شملت الدراسة أيضا محاولة فصل المكونات المختلفة لبروتين بياض بيض الدجاج الرود ايلاند باستعمال مرسبات مختلفة للبروتين مثل كبريتات الأمونيون والأسيتون والكحول والحرارة والفرز الفشائى وكانت نتائج هذه الدراسة ما يلى :

يحتوى بياض بيض دجاج الرود ايلاند على عدة أنواع من البروتينات ينضوى تحت لواء كل منها عدة مكونات وهى :

(أ) نوع الأوفالبيومين وينفصل عند تركيز ٥٢٪ كبريتات أمونيوم وأمكن تقريده الى اربعة مكونات .

(ب) نوع الكونالبيومين ويفصل عند تركيز ٥٧٪ كبريتات أمونيوم ويحتوى على مكون واحد .

(جـ) نوع الأوفاميوكويد وهو بروتين لا يتجمد بالحرارة وينفصل عند تركيز ٦٥٪ كبريتات أمونيوم وأمكن تفريده الى خمسة مكونات الا أنها تكون متراكبة وموجودة فى منطقة الأوفالبيومين .

(د) عند تركيز ٦٠٪ كبريتات أمونيوم أمكن فصل ٣ مكونات جديدة لم يسبق لأحد تسميتها أو تحديد موضعها على ورق التفريد .

(هـ) باستعمال تركيزات من الكحول والأسيتون ، كبريتات الأمونيوم أمكن فصل النوع الأخير من البروتينات المكونة للبياض وهو الجلوبيولين وأمكن تفريده الى ثلاثة مكونات اثنين منها متراكبة وموجودة فى منطقة الكونالبيومين .

* قسم الانتاج الحيوان (فرع التغذية) كلية الزراعة . جامعة القاهرة .