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BIOCHEMICAL STUDIES OF EGG YOLK PROTEINS

By

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1.—Paper electrophoresis technique was used for analysis of egg yolk proteins.

2.—Comparison of the egg yolk water soluble protein in different varieties of birds was made.

3.—Fractionation of egg yolk water soluble proteins by ammonium sulphate was achieved.

4.—Amino acids of the different proteins in the egg yolk were estimated qualitatively.

For a long time egg white and egg yolk were considered important constituents of human diet. As some work on the chemistry of proteins from different sources was undertaken in this laboratory and extensive research was done one egg white protein, therefore it was thought of great importance to carry out a survey on the chemistry of egg yolk proteins.

Sugano (1958), showed that egg yolk proteins cotained phosvitin 8.5%, α - lipovitellin 20.3%, β - lipovitellin 43.2%, γ - component 3.0%, livetin subfraction L_1 2.2, L_2 8.2, L_3 3.2% and others 10.8%.

Burlely and Cook (1961) showed that analysis on solution of egg yolk granules yielded 70% α- and β-lipovitellin (1:1.8), 16% phosvitin and 12% low density lipoprotein.

Martin *et al.*, (1963), isolated the 3 lipoproteins of egg yolk and β-lipovitellin and the low-d-fraction.

Shepard and Hottle (1949), reported that livetin fraction using electrophoretic analysis showed three principal peaks.

Young and Phenney (1951), used several methods to fractionate the yolk proteins of the eggs.

The present work was undertaken in order to study the different proteins of egg yolk.

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Materials and Methods

In these studies hen eggs of different species belonging to different phylogenetic orders were used. Egg samples were provided by the High Agricultural Institute Poultry Farm of Kafr-el-Sheikh.

Preparation of different egg yolk protein

Fresh unbroken yolks were rolled over filter paper to remove all adhering materials. The cleaned egg yolk was then pierced and diluted 10% sod. Chloride solution (1 : 3 V/V). This mixture was filtered and the lipid contents were removed by extraction with ethyl ether. After extraction for several times the clear aqueous solution was subjected to paper electrophoresis.

Paper electrophoresis technique

Method described by Durrum (1950) was used utilizing pyridine/acetic buffer at pH 5.5, and 0.1 M sod. carbonate/bicarbonate buffer solution pH 9.8. The papers were stained with azocarmine dye.

Quantitative determination of the protein bands

Protein bands which were localized were estimated by a Model MGF Belin densitometer. Measurements were recorded graphically in the form of a curve.

Preparation of different identified fractions from egg yolk

1. Lipovitellin : The method of Charagoff (1942), was used.
2. Lipovitellenin : The method of Shepard and Hottle (1949), was used.
3. Phosvitin : The method of Joubert and Cook (1958), was used.
4. Livetin : The method of Shepard and Hottle (1949), was used.

Paper Chromatography technique

Paper chromatographic method was used for determination of the amino acids content of egg yolk protein fractions after hydrolyzing with HCl (6 N.) and NaOH (5 N.) for 20 hrs. Two dimention chromatography were run with butanol-acetic-water (4:1:5) followed by phenol-water (8:2).

The apparaties and working details were described by Dent (1948). Chromatograms were run by the descending technique.

Results

It has been found that egg yolk proteins consisted mainly of lipovitellin, lipovitellenin fraction the phosvitin and the water soluble protein livetin. Using electrophoretic technique it was found that a good separation of these proteins was obtained by using the following procedure.

The whole egg yolk of hen was diluted with saturated NaCl (1 : 3 V/V), extracted with cold Et_2O and dialyzed against distilled H_2O, a precipitate was dissolved in 10% NaCl and subjected to electrophoretic analysis. The result was a presence of three bands as shown in fig. 1. These bands represented the lipovitellin fraction.

The same electrophoretic pattern could be obtained for the lipovitellenin fraction. Such fraction was previpitated from egg yolk by dilution with two volumes of 0.85% NaCl and extrating with ether.

On evaporating the residual ether, a further precipitate was appeared which consist of Lipovitellenin.

This latter precipitate when dissolved in 10% NaCl, extracted by cold chloroform and centrifuged a clear solution was obtained. Using electrophoretic analysis, the presence of two bands was shown in fig. 2 which may be the phosvitin fraction.

It was worth while indicating that some of the egg yolk proteins are water soluble. So it was desirable to prepare that protein by the following method: The egg yolk was diltued with water (1 : 3 V/V) and dialyzed against water. The precipitate formed was centrifuged and was discarded. The supernatant was examined electrophoretically. The result showed the presence of seven distinct bands representing the livetin fraction fig. 3 numbered from 1 to 7.

Comparison of egg yolk livetin fraction in different varieties of birds :

An experiment was set to study the livetin electrophoretic pattern of different bird varieties in U.A.R. It has been previously shown in this study that hen livetin pattern revealed the presence of seven bands as shown in fig. 3. On the other hand the livetin electrophoretic pattern of Turkey, Sudani-duck, Bekini duck and Goose showed difference in mobility but sinmilarily in having five bands in each pattern as shown in figs.: 5, 6, 7 and 8.

Quantitive determination of the water soluble protein Livetin of different eggs yolks :

The densitometer was used to measure the color of the bands located on electrophoretic patterns. This color density was recorded in a shape of a curve. The curve was divided into areas under each peak.

The area under each peak relative to the total area was mesaured by a planimeter. From these measurements the percentage of each protein was determined as shown in figs. :4, 9, 10, 11, 12 and 13.

Fractionation of hen egg yolk Livetin :

The ammonium sulphate fractionation technique was applied for the fractionation of the egg yolk water soluble proteins. Several fractions were obtained. At 31 % saturation, a precipitate was separated, dissolved in the original buffer and subjected to paper electrophoresis. An undistinct spreading band was obtained from the starting point to the end of band 7 of the original livetin electrophoretic pattern as shown in fig. 14. At 39 % saturation, traces of the three bands 3,4 and 5 in addition to the formentioned spreading band was obtained as shown in fig. 15. At 50% saturation a fraction indicating the presence of band 3, 4 and 5 in addition to the small amounts of the spreading band was obtained as shown in fig 17.

At 65% saturation, the band 1 and 2 some of band 3 were spreaded as a fraction as shown in fig. 16.

At 85 % saturation a fraction representing band 6 and a band in the place of band 5 was separated as shown in fig.17 b.

Qualitative determination of amino acids of egg yolk proteins :

The analysis was performed on the hydrolyzates of proteins from the egg yolk of hen. A summary of the chromatographic results is given in Table I.

TABLE 1.—AMINO ACIDS OF EGG YOLK PROTEINS

Posvitin	Lipovitellin	Livetin
isoleucine	isoleucine	—
leucine	leucine	leucine
argenine	argenine	argenine.
threonine	threonine	—
aspartic acid	aspartic acid	aspartic acid.
lysine.	lysine.	lysine.
cysteine.	—	cysteine.
glutamic acid	glutamic acid	glutamic acid.
phenylalanine	phenylalanine	phenylalanine.
tyrptophane	tryptophane	tyrptophane.
alanine	alanine	alanine.
methionine	methionine	methionine.
valine	valine	—
histidine	histidine	histidine.
tryosine.	tyrosine.	tryosine.
glycine	glycine	—
proline	proline	proline.
serine.	serine.	—

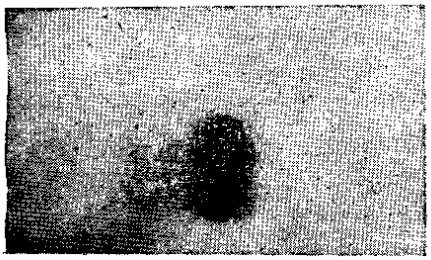


Fig. 1_Lipovitellin & Lipovitellenin.

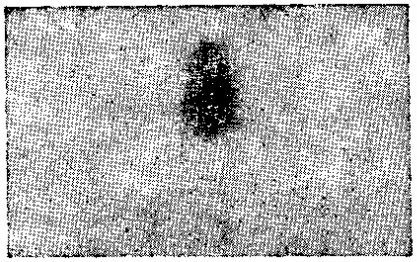


Fig. 2_a) Lipovitellin. b) Phosvitin

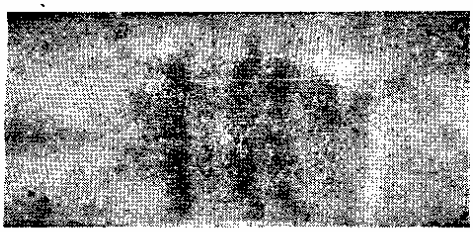


Fig. 3_ Livetin fraction

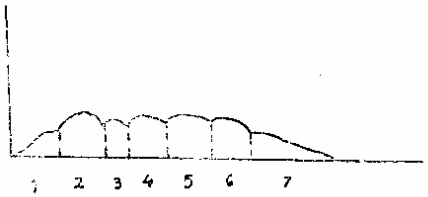


FIG. 4—Electrophoretic curve of Livetin

Electrophoretic patterns of egg yolk proteins of hen. (for Rhode Island Red)

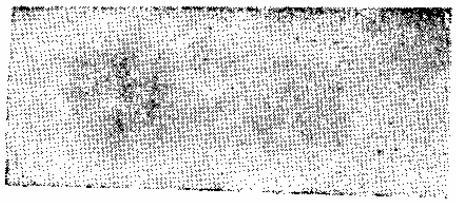


Fig. 5_Turkey egg yolk Livetin

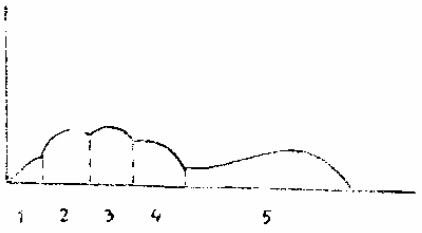


FIG. 9.—Electrophor. curve (Turkeys)

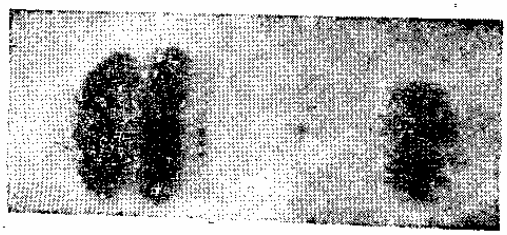


FIG. 6 —Sudani duck ivetin

FIG. 10.— Electrophor. curve (Sudani ducks)

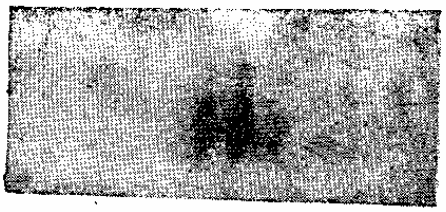


Fig. 7_Bekini duck Livetin

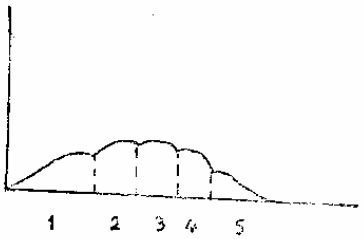


FIG. 11.—Electrophor. curve (Pekin ducks)

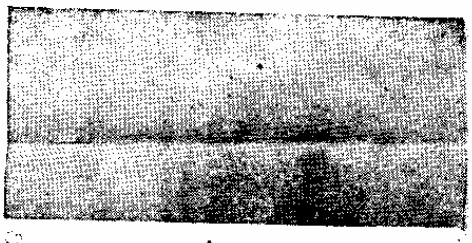


Fig. 8_Goose Livetin

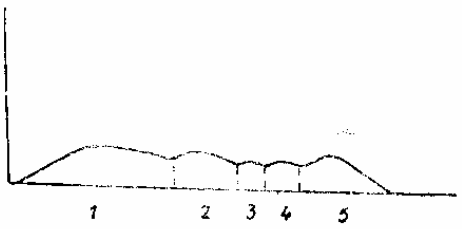
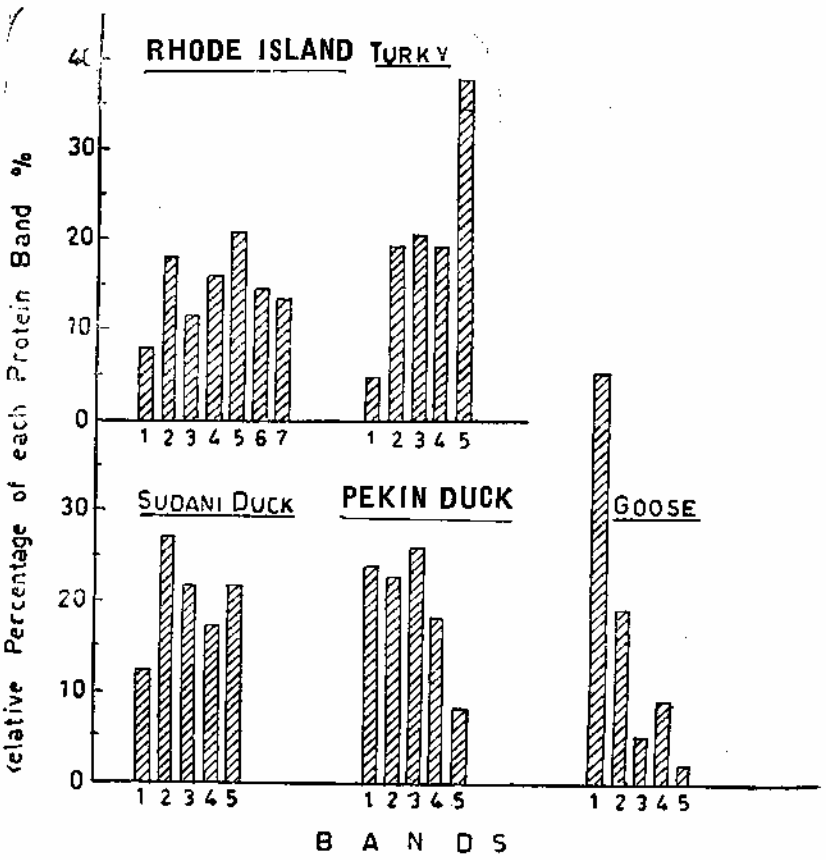


FIG. 12.—Electrophor. curve

Electrophoretic patterns and curves of Livetin bands.



13_Percentage of Livetin Fractions in Different Birds

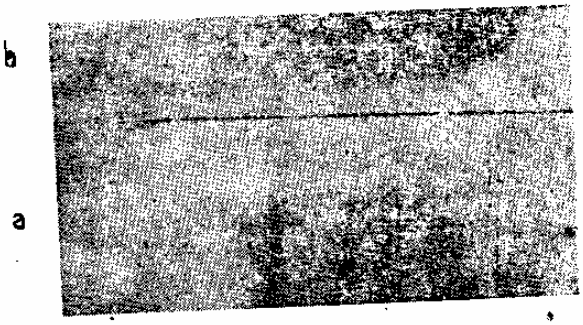


Fig. 14_a) Hen egg yolk livetin

b) Fraction (31% sat. amm. sulphate)

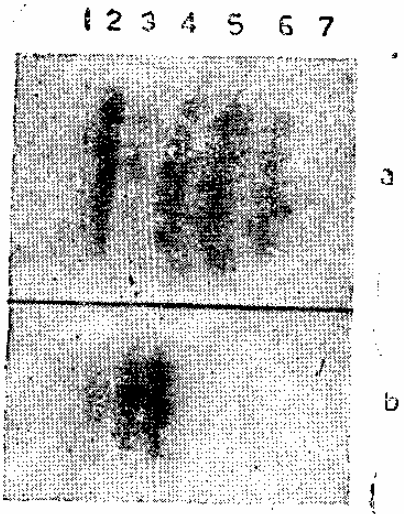


Fig. 15_a) Egg yolk Livetin

b) 39% sat. amm. sulphate fraction.

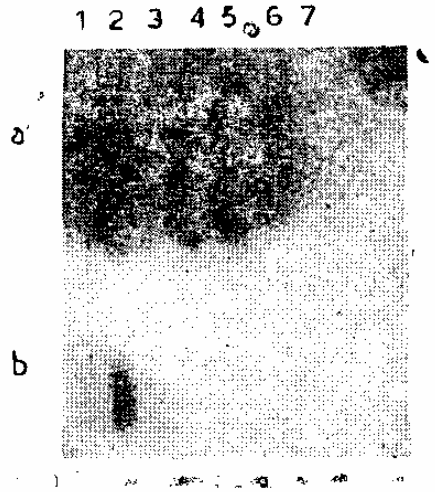


Fig. 16_a) Egg yolk Livetin

b) 65% sat. aomm. sulphate fraction.

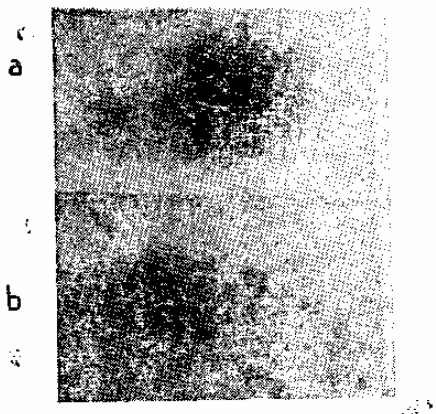


Fig. 17_a) 50% Am. sulp. fraction

b) 85% amm. sulphate fraction.

Electrophorotic patterns of hen's egg yolk livetin after fraction with saturated amm. sulphate.

Discussion

The comparative biochemistry of egg proteins has so far attracted only occasional and identical attention.

From the results represented in this paper it is possible to make a few comparison largely related to the egg yolk proteins of a few type of birds.

The work described here compromised an examination by paper electrophoresis of the soluble proteins of egg yolk and their amino acids constituents. The most striking fact emerging from this work is the considerable difference in the electrophoretic diagram of different phylogenetic orders.

The differences are not merely of degree since some quantitatively important components of the proetins found in eggs are present in some species but compleately absent from others. Yuki and Fuju (1962) and Sugano (1958) reported the same results.

Fractionation of egg yolk proteins was achieved by different methods. Egg lipoproteins, named lipovitellin and lipovitellenin, phosvitin and livetin were fractionated. The amino acids of these bands were analyzed.

The water soluble protein livetin seemed dificient in the amino acids isoleucine, threonine, valine, glycine and serine.

Also the water soluble protein livetin was fractionated and it was found to contain seven distint bands. Trials for the separation of these bands utilizing the ammonium sulphate precipitation technique were achieved.

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دراسة كيميائية حيوية عن بروتينات صفار البيض

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الملخص

أجريت هذه الدراسة على بروتينات صفار البيض المأخوذ من دجاج الرود ايلاند والرومى والبط السودانى والبط البكينى والأوز بغرض معرفة مكوناتها بطريقة التفريد الكهربى على رق الترشيح ودلت النتائج على ما يلى :

أمكن الحصول على تفريد واضح لبروتينات صفار دجاج الرود ايلاند ووجد أنه يتكون من أربعة مكونات هى : الليبوفيتيللين ويحتوى على ثلاث وحدات بروتينية ليبوفينتيلثين ويحتوى على ثلاث وحدات بروتينية ، فوسفتين ويحتوى على ثلاث وحدات بروتينية . والمكون الرابع وهو يمثل بروتينات الصفار القابلة للذوبان فى الماء ويسمى (ليفتين) وقد أمكن تفريده الى سبع وحدات بروتينية .

تم التعرف على الأحماض الأمينية الموجودة فى كل مكون بروتينى بعد فصله على حالة نقية ووجد احتوائه على أحماض ايزولوسين ـ لوسين ـ أرجنين ـ تريونين ـ حامض الاسبارتيك ـ ليسين ـ سستين ـ حامض جلوتاميك ـ فينايل الانين ـ تربتوفان ـ الانين ـ مثيونين ـ فالين ـ هستيدين ـ تيروزين ـ جليسين ـ برولين ـ سيرين . الا أن المكون ليفتين تنقصه أحماض ايزولوسين ، فالين ، جليسين ، سيرين .

وقد شمل البحث ايضا فصل المكون ليفتين من صفار بيض الرومى والبط السودانى والبكينى والأوز وعند تفريده أعطى خمسة وحدات بروتينية فقط بدلا من سبعة كما فى دجاج الرود ايلاند ، وكانت هذه الوحدات تختلف عن بعضها فى الكمية وفى مكان الهجرة .

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