

The Phosphorus as Lecithin, Protein and Inorganic Compounds in the Yolk of Egg.

In analyzing the Gertrude Elma Hole.

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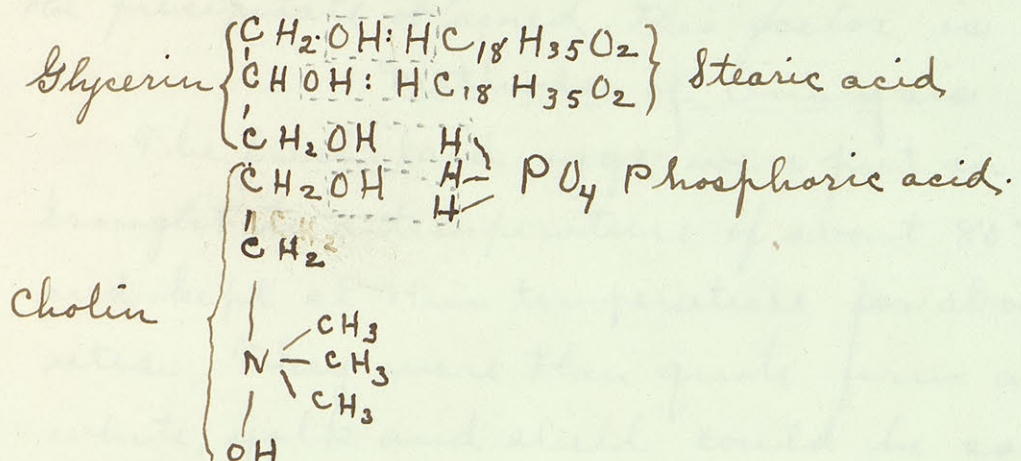
The Phosphorus as Lecithin, Protein and Inorganic Compounds in the Yolk of Egg.

In making the chemical analysis of eggs we find that the white is composed of water and protein, or almost pure albumen, and a minute quantity of inorganic salts; but the yolk is quite different, it having about 50% of water and the remainder solid matter which is protein, ether extract and inorganic compounds. In analyzing a portion of this solid yolk we find that there exists in it a considerable portion of phosphorus. The question then occurs, "How much of this phosphorus exists as organic and in what form does it exist?"

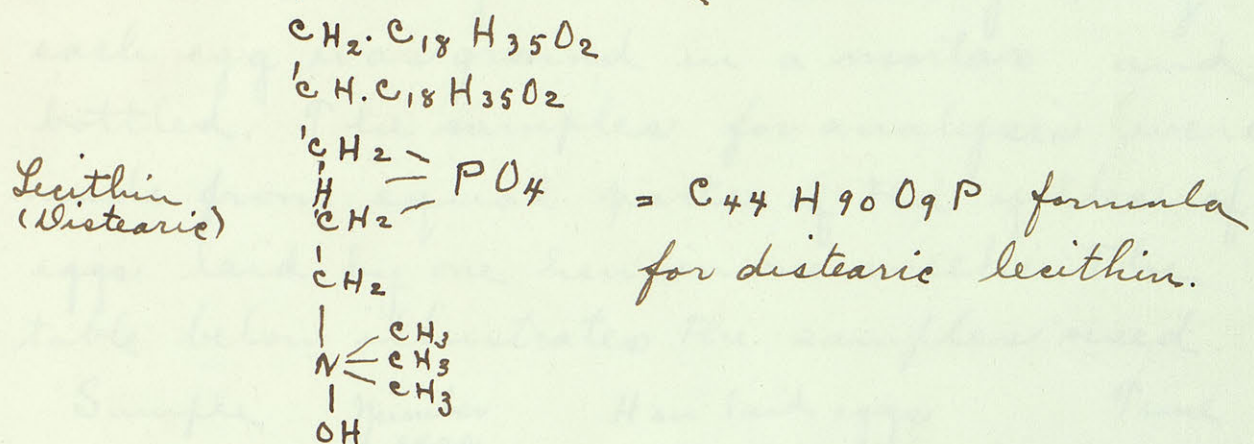
There is a substance in the yolk of egg known as lecithin which as yet is not thoroughly understood but whose importance can be judged from the summary which W. Maxwell gives in his valuable work on the subject of egg-analysis. "Lecithins are a source of the phosphorus required by the animal organism in the formation of bone. These bodies are also the medium by which the phosphorus present in vegetable organisms is received by the animal organism." This

investigations have shown finally "That the lecithins exercise the function of receiving phosphorus from minerals and bestowing it in the processes of the animal organism in the formation of bone. These bodies are thus the medium through which the element performs its movements, through the vegetable and into the animal kingdom."

Lecithin is a complex compound which is related to the fats in composition. It is a hygroscopic wax-like substance which swells up in water, is very soluble in alcohol, ether, chloroform, and oils. It is found not only in the animal tissue, as the brain, nerves, and yolk of egg, but also in the vegetable world. It occurs in nearly all animal and vegetable cells, fluids and juices. As it is so widely distributed we must acknowledge its physiological importance. Lecithin is a combination of two molecules of certain organic acids, glycerophosphoric acid, and a complex substance called cholin. Below is given the combination and structural formula of the distearic lecithin. Distearic lecithin is the lecithin in which two molecules of stearic acid is in combination with glycerophosphoric acid and cholin.



In the interaction 4 molecules of water (H_2O) are eliminated and we have



The cholin and glycerophosphoric acid may be in combination with palmitic acid or oleic acid and it has been found by E. Schulze in his analysis of plants that the lecithin does occur as other than the distearic.

The distearic lecithin is the lecithin considered present in the yolk of egg and is used for the calculations. The formula is $\text{C}_{44}\text{H}_{90}\text{O}_9\text{P}$ and the molecular weight is 807. The Hoppe-Seyler factor for converting magnesium pyrophosphate, which is produced by igniting

The precipitate obtained, This factor is 7.2703

Methods of Analysis

The fresh-laid eggs were put in cold water brought to a temperature of about 80° Centigrade, and kept at this temperature for about 45 minutes. They were then quite firm and the white, yolk and shell could be easily separated and moisture determinations made of the white and yolk. The dried yolks of each egg was ground in a mortar and bottled. The samples for analysis were made from equal parts of the yolks of eggs laid by one hen in one week. The table below illustrates the samples used.

Sample	Number of eggs	Hen laid eggs	Time
1 - - - - -	5 - - - - -	White Leghorn - - - - -	1st week
2 - - - - -	5 - - - - -	Plymouth Rock - - - - -	1st week
3 - - - - -	4 - - - - -	White Leghorn - - - - -	2nd week
4 - - - - -	6 - - - - -	Plymouth Rock - - - - -	2nd week

The phosphorus determinations were made in accordance with the "Official Methods of Analysis". The extractions were made by alcohol-ether as done by Maxwell (American Chemical Journal. Vol. 13 + Vol. 15).

The solutions and preparations necessary for the analyses which were made, are given with the method of preparation and

composition —

- (1) Magnesia Mixture. Dissolve 110 grams of crystallized magnesium chloride in a small quantity of water, add 280 grams ammonium chloride, 700 c.c. of ammonia of specific gravity 0.96 and enough water to make up to 2 liters.
- (2) Molybdic Solution. Dissolve 100 grams of molybdic acid in 400 grams or 417 c.c. of ammonia, specific gravity 0.96 and pour the solution thus obtained into 1250 c.c. of nitric acid, specific gravity 1.20. Decant from yellow precipitate which may have formed after standing in a warm place for several days.
- (3) Ammonia. Ammonia of standard strength diluted with water until of specific gravity 0.96. This is for use in making molybdic solution, magnesia mixture and for adding to precipitate caused by magnesia mixture. More ammonia is diluted to 2½% and is used for washing the magnesium precipitate — the precipitate of the phosphorus by the magnesia mixture.
- (4) Ether Absolute. Ether is placed over sodium wire thus decomposing any water and alcohol which may be present. The ether is then distilled to free it from any oil which may have been introduced by means of the sodium.

(5) Alcohol Absolute. Alcohol of about 94% was heated over lime with reflux condenser for several hours. Then it was distilled off and put in dry bottle. It was nearly absolute.

Weighings were made of 1. gram of the air dry sample, which, when dried in steam oven, weighed from .03 to .05 grams less.

These charges were each digested in a Kjeldahl flask with concentrated sulphuric acid and potassium sulphate for several hours or until all the organic matter was decomposed and the residue was of a light lemon color, 100 c.c. of water was then added and this boiled. After cooling, the solution was transferred to 250 c.c. flask and made up to mark. An aliquot portion ($\frac{2}{5}$) was taken for each of duplicate determinations, neutralized with ammonia and cleared with nitric acid, 15 grams of ammonium nitrate was added, the beakers containing these aliquots were put on hot plate, then 20 c.c. of molybdic solution was added to each and they were allowed to stand on hot plate for several hours. Each was then filtered and the precipitate on the filter was dissolved with hot water and ammonia, washed into a beaker and when cool it was nearly

neutralized with hydrochloric acid and when again cool 10 c.c. of magnesia mixture was added very slowly from a burette, while stirring the solution vigorously, in a few minutes 30 c.c. of ammonia (specific gravity 0.96) was added to each beaker and they were left standing several hours. The 2½% ammonia was used for washing the precipitate obtained by the above treatment of the solution.

The precipitate was then ignited to whiteness in a muffle and weighed as magnesium pyrophosphate ($\text{Mg}_2\text{P}_2\text{O}_7$)

In the following table the weights of the 1. gram charge of the air dry samples, when thoroughly dried in steam oven, are given, also the duplicate weights of the magnesium pyrophosphate obtained for each sample.

Sample	$\text{Mg}_2\text{P}_2\text{O}_7$	Charge dried.
1 - - - - -	.0164 grams .0163 - " - - - - -	.9741 grams
2 - - - - -	.0190 - " .0189 - " - - - - -	.9769 " .
3 - - - - -	.0170 - " .0166 - " - - - - -	.9528 " .
4 - - - - -	.0157 - " .0159 - " - - - - -	.9553 " .

The weight of magnesium pyrophosphate ($\text{Mg}_2\text{P}_2\text{O}_7$) upon being multiplied by the phosphorus factor .2784 and divided by the aliquot factor .4 gave the number of grams of phosphorus in the charge taken. Sample 1.-1. gram of

air dry sample equals .9741 grams of dried sample.

By dividing in each case the average of the weights of the phosphorus by the charge we find the per cent of phosphorus in the dried yolk.

The per cent that the dried yolk is of the whole yolk is about 50. Given 1.18 as per cent in dried yolk, 100 as whole yolk, 51.15 as dried yolk, by proportion $100:51.15::1.18:X$, we find the per cent of phosphorus in the whole yolk.

The per cent of dried yolk in whole yolk, the per cent of phosphorus in dried yolk, and the per cent of phosphorus in the whole yolk are here given.

Sample	% dried yolk	% Phosphorus in dried yolk.	% phosphorus in whole yolk.
1 - - - - -	51.15 - - - - -	1.18 - - - - -	.604.
2 - - - - -	51.67 - - - - -	1.35 - - - - -	.700.
3 - - - - -	50.09 - - - - -	1.24 - - - - -	.620.
4 - - - - -	51.08 - - - - -	1.16 - - - - -	.600.

In the determination of phosphorus as lecithin, charges of 5 grams of air dry sample were taken. These when dried in steam oven lost from .12 to .22 grams in weight.

These charges were extracted with ether for about 2 hours then they were taken out and ground to a powder in a mortar, the extraction with ether was then continued.

for the remainder of the 15 hours. The extraction was then dried and weighed this being the "1st ether extract." The charges were then extracted with absolute alcohol for 10 hours, then the extract was absorbed by fat-free paper, dried and re-extracted with ether for 8 hours, this being the "2nd ether extract." The alcohol dissolves the lecithin which is not soluble in the ether but after solution in alcohol the lecithin becomes soluble in ether and upon dissolving in the ether eliminates any inorganic substances which may have been dissolved by alcohol. The extracts are as follows.

Sample.	Charge - dried	1st ether extract	2nd ether extract	Total ether extract
1 - - -	4.8707	3.0538	.1506	3.2044.
2 - - -	4.8845	3.1681	.2076	3.3757.
3 - - -	4.7642	2.9883	.0912	3.0795.
4 - - -	4.7763	3.0250	.1651	3.1901.

Each of the total extracts, which contained all of the lecithin, was then transferred by dissolving in ether, to a Kjeldahl flask, the ether was distilled off, and the residue, or extract, was then treated with concentrated sulphuric acid and potassium sulphate and digested for several hours as in the determination for total phosphorus. This was

then diluted to 250 c.c. and an aliquot ($\frac{2}{5}$) was taken. This required about 50 c.c. molybdic solution and about 25 c.c. magnesia mixture. The amounts of magnesium pyrophosphate ($Mg_2P_2O_7$) for the samples were as follows.

Sample	Charge dried yolk	Charge of total ether extract	$Mg_2P_2O_7$ in whole charge average of duplicates
1 - - -	4.8707 -	3.2044 -	.1051.
2 - - -	4.8845 -	3.3757 -	.1179.
3 - - -	4.7642 -	3.0795 -	.0952.
4 - - -	4.7763 -	3.1901 -	.1065.

From the above figures we have the $Mg_2P_2O_7$ from the given charge of the dried yolks.

We have the percent of dried substance in each yolk used in sample. From this and the weights of each individual yolk we calculate the weight in grams of dried substance in each yolk in sample, and the average of these is taken as the weight of dried yolks in the sample. Then find the average weight of the whole yolks of the eggs in each sample. And the average weight of the eggs in each sample.

The weight of each egg taken is the sum of the weights of the yolk, white and shell of that egg, after being cooked, separated and weighed - thus the egg taken is from 3 to 4 grams less in weight than the original egg.

Table shows figures obtained by calculation as above explained.

Sample	Wt. of dried yolk.	av. weight of whole yolks.	av. weight of eggs.
1 - - - - -	9.33 grams	18.24 grams	52.42.
2 - - - - -	10.24 "	19.82 "	52.77.
3 - - - - -	8.96 "	17.88 "	52.78.
4 - - - - -	9.01 "	17.64 "	50.73.

(Figures from sample 1 used in example)

Since .1051 grams $Mg_2P_2O_7$ (given in preceding table) is obtained as phosphorus of lecithin from 4.8707 grams dried yolk, then from 9.33 grams or the whole dried yolk we find by proportion—
 $4.8707 : 9.33 :: .1051 : x$ $x = .2014$ grams or the amount of $Mg_2P_2O_7$ produced from the phosphorus of lecithin in the whole dried yolk, hence in the whole yolk and in the whole egg for it (lecithin) exists in just the dried yolk, or the solid substance of the yolk. By multiplying ^{wt. of $Mg_2P_2O_7$} by the Hoppe-Seyler factor (7.2703) we find the whole amount of lecithin in the egg.
 This weight divided by (1) the weight in grams of the whole yolk, gives the per cent of lecithin in the whole yolk, and (2) by the weight in grams of the whole egg gives the per cent of lecithin in the whole egg—thus
 (table on following page)

Sample	lbs. $Mg_2P_2O_7$ from dried yolk.	% of lecithin in whole yolk.	% of lecithin in whole egg.
1 - - -	.2014 grams	8.03 - - -	2.79.
2 - - -	.2458 "	9.01 - - -	3.39.
3 - - -	.1730 "	7.03 - - -	2.38.
4 - - -	.2010 "	8.28 - - -	2.88.

By multiplying the number of grams of $Mg_2P_2O_7$ by the phosphorus factor (.2784) we find the number of grams of phosphorus in the whole yolk - i.e. the amount of phosphorus that exists as lecithin. By dividing by the weight of yolk the per cent of phosphorus as lecithin in the whole yolk is obtained and by dividing by the weight of the whole egg the per cent of phosphorus as lecithin in the whole egg is obtained.

Sample	grams phosphorus.	% of phosphorus (as lecithin) in whole yolk	% of phosphorus (as lecithin) in whole egg.
1 - - -	.0563 - - -	.3085 - - -	.1074.
2 - - -	.0687 - - -	.3465 - - -	.1302.
3 - - -	.0483 - - -	.2704 - - -	.0916.
4 - - -	.0563 - - -	.3192 - - -	.1110.

In another table we find the total phosphorus in the whole yolk.

A comparison of the total phosphorus in the whole yolk and the phosphorus as lecithin in the whole yolk, is given in the following table -

Sample	Total phosphorus in whole yolk	Phosphorus as lecithin in yolk.
1 - - - - -	.604 - - - - -	.309 .
2 - - - - -	.700 - - - - -	.347 .
3 - - - - -	.620 - - - - -	.270 .
4 - - - - -	.600 - - - - -	.319 .
Average .631 - - - - -		.311 .

$\frac{.311}{.631} = .492$ or very nearly $\frac{1}{2}$ of the phosphorus in the yolk exists as lecithin.

The remainder (or $\frac{1}{2}$) of the phosphorus exists as protein and inorganic compounds.

The egg yolks when analyzed had been dried and bottled, not in air tight bottles, for one year, but they had not, seemingly, decomposed at all.



S.E. Hale making analyses