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**COTTONSEED MEAL IN POULTRY FEED**

# Gossypol-Cephalin Compound from Fresh Eggs of Hens Fed Cottonseed Meal

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**The cephalin fraction of egg yolks from hens fed gossypol is yellow in color, and possesses an absorption spectrum with maxima at 380 and 400 mμ. Gossypol was identified in the cephalin fraction after oxalic acid hydrolysis. The reaction of gossypol with ethanolamine and with ethylamine resulted in products having absorption spectra that were almost identical with the absorption spectrum of gossypol egg cephalin. It is concluded that the aldehyde groups of gossypol condense with the primary amino groups of phosphatidylethanolamine to form a Schiff base which is present in the egg.**

THE METABOLISM OF GOSSYPOL in the animal body has not yet been elucidated, but there is abundant evidence that the feeding of this toxic yellow pigment to laying hens results in the producduction of egg yolks which may discolor during storage (*7, 9, 10*). Swensen Fieger, and Upp (*11*) concluded that gossypol is absorbed unchanged from the intestinal tract of the hen, and is deposited in the egg in the free form. Evidence presented in support of this conclusion was rather indirect, as free gossypol has never been isolated from egg yolk.

Egg yolks from hens fed gossypol contain a distinctive yellow component which is insoluble in acetone, but is soluble in hexane-acetone 3 to 1 by volume (*5*). Spectrophotometric estimation of the amount of this component in the eggs reveals a direct relationship with the amount of gossypol fed. In this paper, evidence is presented to show that the yellow component is a compound of gossypol with cephalin.

## *Experimental*

**Preparation of Cephalin.** Cephalin was prepared from normal eggs and from eggs of hens fed cottonseed meal as a source of gossypol (hereinafter called "gossypol eggs"). Fresh egg yolks were exhaustively extracted with acetone (*5*), followed by three extractions with 3 to 1 hexane-acetone. The hexane-acetone extracts were combined in two separate flasks, and evaporated under reduced pressure. Cephalin was prepared from the fatty residues by using the following procedure, which is a modification of that developed by Folch (*4*). The solvent volumes are expressed on a per egg basis.

The fatty residue was extracted twice with 5-ml. portions of 95% ethyl alcohol, and once with 2 ml. of petroleum ether (boiling range 30° to 60° C.). The petroleum ether extract was evaporated under reduced pressure, and the resulting residue was dissolved in 1 ml. of diethyl ether. This mixture was allowed to remain at −10° C. overnight, and the appreciable white precipitate which formed was discarded. The supernatant fluid was diluted with 1 ml. of diethyl ether, and the cephalin was precipitated with 10 ml. of 95% ethyl alcohol, which was added slowly during stirring. After remaining at room temperature for an hour, the sample was centrifuged at about 2000 r.p.m. for 5 minutes, and the supernatant fluid was discarded. The yield was approximately 43 mg. per egg. The cephalin was stored at −10° C. in solution with diethyl ether, or as a precipitate under acetone. It was more stable in acetone. The normal egg cephalin was almost white in color, while the gossypol egg cephalin was yellow. Attempts to fractionate the cephalin by the method of Folch (*4*) were not successful.

**Hydrolysis of Cephalin.** Cephalin which had been stored in diethyl ether

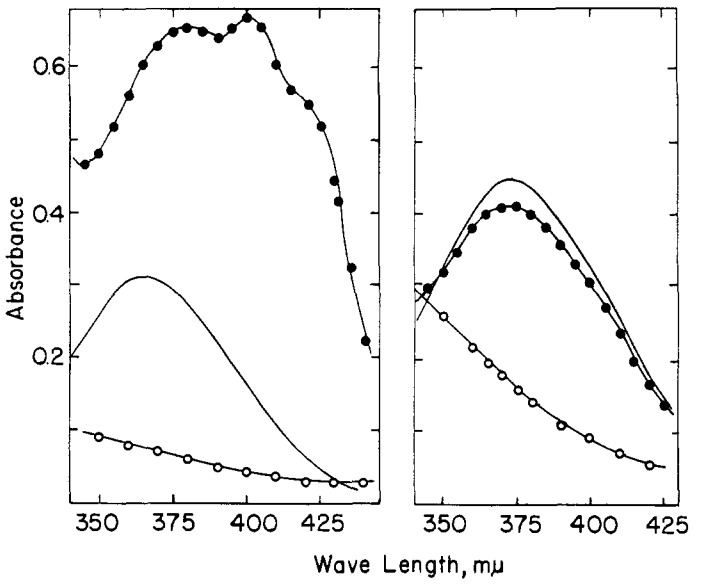



Figure 1. Absorption spectra

*Left.* Cephalin
○ From one normal egg
● From one gossypol egg
— 0.0092 mg. per ml. of gossypol

*Right.* Hydrolyzates
○ Normal egg cephalin
● Gossypol egg cephalin
— Gossypol treated in same manner

at −10° C. for one month was used in this experiment. The ether was evaporated at room temperature by a stream of nitrogen, and the cephalin was dried for 2.5 hours in a vacuum oven at 60° C. Then 300 mg. of normal egg cephalin and 300 mg. of gossypol egg cephalin were weighed into 50-ml. volumetric flasks, and hydrolyzed with oxalic acid according to the method developed by Pons, Hoffpauir, and O'Connor (*8*) for determination of bound gossypol in cottonseed meal. This method was modified, in that the solutions were diluted to a final volume of 50 ml. instead of 100 ml. The hydrolysis was allowed to proceed for 10 hours at 70° C. A 0.7-mg. sample of pure gossypol was also subjected to the same treatment. The gossypol egg cephalin hydrolyzate was diluted 1 to 1 with the blank before absorption spectra were made.

**Reaction of Gossypol with Ethanolamine** (2-Aminoethanol). Materials used consisted of a 1 to 100 dilution of ethanolamine at pH 7 in absolute ethyl alcohol, and an acetone solution containing 0.92 mg. of gossypol per ml.; 9.9 ml. of the ethanolamine solution were mixed with 0.1 ml. of the gossypol solution, and the reaction which took place at room temperature was followed by determining the absorption spectrum at 10-minute intervals for 70 minutes.

**Reaction of Gossypol with Ethylamine.** A 70% aqueous solution of ethylamine was diluted as above, and made to react with gossypol in the same manner.

**Spectrophotometry.** All absorption data were obtained with a Beckman Model B spectrophotometer, using a 1-cm. Corex cell.

## Results

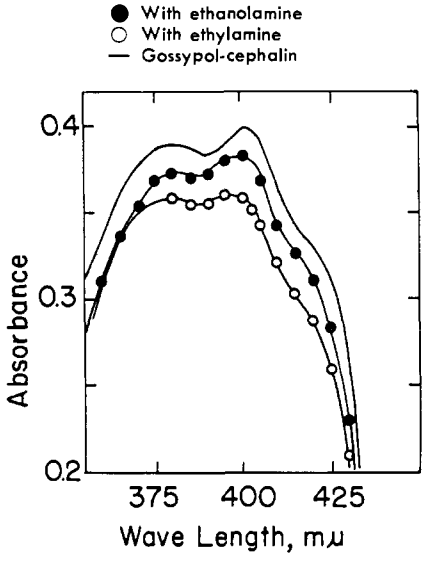
Figure 1 (left) shows the absorption spectra of normal egg cephalin, gossypol egg cephalin, and gossypol. Gossypol egg cephalin has peaks at 380 and 400 mμ, while gossypol has a peak only at 365 mμ. Normal egg cephalin exhibits only weak absorption over the range studied.

The results of the oxalic acid hydrolysis are shown in Figure 1 (right). The gossypol egg cephalin hydrolyzate exhibits an absorption maximum at approximately 373 mμ, a result identical with that obtained with pure gossypol. In contrast, the absorption of normal egg cephalin hydrolyzate decreases rapidly in this range.

The gossypol-ethanolamine reaction (Figure 2) proceeded to completion after 45 to 60 minutes at room temperature, and was stable for over 5 hours at that temperature. The reaction product has absorption maxima at 380 and at 400 mμ, the same as gossypol egg cephalin.

The reaction between gossypol and ethylamine (Figure 2) proceeded to completion after 30 to 40 minutes at room temperature, and was stable for another hour. The absorption spectrum of the product has maxima at 380 and 395 mμ, and closely resembles the absorption curve of gossypol egg cephalin.

Figure 2. Absorption spectra of reaction products of gossypol




## Discussion

When gossypol is present in egg yolk, it is present not in free form as postulated by Swensen, Fieger, and Upp (*11*), but in bound form. Chargaff, Ziff, and Rittenberg (*2*) found that egg cephalin consists almost entirely of phosphatidylethanolamine. As gossypol readily reacts with amino groups (*1, 3, 6*), it is concluded that the primary amino groups of egg cephalin condense with the aldehyde groups of gossypol to form a Schiff base.

Unpublished results from this laboratory have also shown that egg yolk protein contains bound gossypol. The gossypol, which is probably attached to free amino groups of the protein, can be released and identified by oxalic acid hydrolysis (*8*) followed by spectrophotometry.

These results indicate that amino groups play an important role in the metabolism of gossypol by the laying hen.

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