or absence as compared with the diet is thus an indication of the extent to which the insect controls its precise pattern of haemolymph-free amino acids.

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A simplified procedure for the preparation of phosvitin and vitellin*

The two major phosphoproteins of egg yolk are phosvitin and vitellin, the latter being in the form of a lipoprotein complex[1]. MECHAM AND OLCOTT[2] described a procedure for the isolation of phosvitin, containing 10 % phosphorus and accounting for nearly 70 % of total yolk phosphorus. The lipovitellin, first isolated by ALDERTON AND FEVOLD[3], was subjected to a detailed electrophoretic and ultracentrifugal study[4,5] which revealed that this protein is a mixture of several components, involving the total phosvitin of egg yolk and the lipid-bound vitellin. The lipovitellin fraction of ALDERTON AND FEVOLD[3] thus promised to be good starting material for the preparation of both phosvitin and vitellin.

Butanol, which is being widely used to liberate the proteins bound to lipids[6], was employed in the present investigation to disrupt the lipid–protein complex of lipovitellin. This procedure, while rendering vitellin insoluble, incidentally released all the phosvitin into solution from which it could be recovered by isoelectric precipitation. The method adopted was as follows:

Yolks from 50 eggs were freed of adhering white and chalazae, and diluted with two volumes of distilled water. The emulsion was passed through a Sharples centrifuge[3] and the residue was taken up in 500 ml 10 % NaCl. Lipovitellin was precipitated by diluting the salt solution with 7–8 l water. The precipitate was collected by centrifuging, and dissolved in 500 ml 10 % NaCl. The saline extract was treated with 250 ml *n*-butanol and the solution was stirred at room temperature for 1 h after which it was kept in the cold for 24 h. After centrifugation, the top

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butanol layer which was orange in colour was siphoned off carefully. The bottom aqueous layer and the middle layer of thick precipitate were separated by filtration on a Buchner funnel. The precipitate was suspended in 500 ml 10 % NaCl, 250 ml *n*-butanol again added and the process was repeated. The aqueous layers were pooled. The insoluble vitellin was washed with water thrice to free it from NaCl, then repeatedly with acetone till the washings were colourless, finally with ether and then air-dried. The yield was about 20 g.

For the preparation of phosvitin, the combined aqueous extract was shaken twice with 150-ml portions of ether in a separatory funnel, and the ether extracts discarded. The clear solution which was light yellow in colour was acidified with 2 *N* HCl with stirring to pH 1.8 and kept in the cold overnight to complete the precipitation. The gelatinous precipitate was centrifuged off, dissolved in 200 ml water by the addition of 2 *N* ammonia to pH 6.0, and then filtered through a fluted filter paper. The protein was reprecipitated by acidification to pH 1.8, and dissolved as before. The solution was treated with $MgSO_4$ to 0.4 *M* saturation and stirred for 1 h at room temperature. Enough water was added to bring down the concentration of $MgSO_4$ to 0.09 *M*. Phosvitin was precipitated as a sticky mass which settled at the bottom of the beaker when kept at room temperature for few hours. After siphoning off the supernatant, the precipitate was washed 2–3 times with water, suspended in water and dissolved by the addition of 2 g ethylenediaminetetraacetate (disodium salt). The protein solution was dialysed in the cold against several changes of distilled water. Phosvitin was precipitated as before by acidification to pH 1.8. The protein was centrifuged off, washed twice with 50 % acetone and then with pure acetone. After a final washing with ether, the product was air-dried. It was obtained as a light white powder and the yield was the same as that obtained by the procedure of MECHAM AND OLCOTT[2].

Phosphorus and nitrogen analyses of the proteins were carried out by the method of FISKE AND SUBBAROW[7] and KOCH AND MCMEEKIN[8] respectively. Vitellin had a P content of 0.66 % and contained less than 0.05 % of hot alcohol–ether extractable solids. The value for phosphorus is lower than those reported by the earlier workers whose preparations had obviously been contaminated with varying amounts of phosvitin[1,4].

Phosvitin had a N/P molar ratio of 2.65 in close agreement with that obtained for the preparation of MECHAM AND OLCOTT[2]. The present preparation and that of MECHAM AND OLCOTT were examined by paper electrophoresis in buffers of different pH. Both the samples gave a major ninhydrin-positive band, when sprayed with the acidic ninhydrin reagent of LEVY AND CHUNG[9], together with two faint slower-moving components. The most rapidly moving component reacted for phosphate when tested by the method of HANES AND ISHERWOOD[10].

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Differences between oxosteroids in their behaviour towards the Girard reagent

The formation of water-soluble derivatives of oxosteroids by treatment with Girard's reagent T is frequently utilized to effect the separation of these steroids from non-ketonic material in lipid extracts of urine[1,2] and tissues[3]. The free oxosteroids can be regenerated from such derivatives by acid hydrolysis. Recently, this procedure has been adapted to the purification of certain highly labile carbonyl compounds by the introduction of two modifications[4]: the use of a carboxylic resin instead of glacial acetic acid as catalyst for the condensation reaction, and treatment with formaldehyde in neutral solution, replacing acid hydrolysis, as a means of splitting the Girard complexes. It has been suggested[5] that the modified procedure may be applied with advantage to oxosteroids, since it eliminates the risks of causing acetylation of hydroxysteroids and partial destruction of labile compounds.

A number of steroids were subjected to the new method of separation in this laboratory. Crystalline steroids (20–200 μg) were dissolved in 1 ml ethanol containing 20 mg Girard reagent T and 5 mg of the resin Amberlite IRC-50 [H] (Rohm & Haas), and either heated to boiling under reflux for 1 h (Series I), or allowed to stand at room temperature for 12 h (Series II). After filtration and dilution with 10 ml water, the "non-ketonic fraction" was separated by extracting 3 times with an equal volume of ethyl acetate. 2 ml 36 % (w/v) formaldehyde solution were then added to the aqueous phase, which was allowed to stand for 7 h at room temperature before re-extracting with ethyl acetate to obtain the "ketonic fraction A". The aqueous residue was then acidified by the addition of 0.4 ml 10 *N* HCl or of 1.5 ml pyruvic acid, and 5–7 h later extracted with ethyl acetate to yield the "ketonic fraction B". The fractions thus obtained were washed to remove residual acid and formaldehyde and taken to dryness. The steroids were then separated by paper chromatography[6] and determined individually.

The experiments, results of which are summarized in Tables I and II, showed that the steroids examined differed markedly both in the rate of formation of a Girard derivative and in the ease with which these complexes could be split. The 3-oxo group of α,β-unsaturated 3-oxosteroids combined readily with the Girard reagent

Abbreviations: Girard's reagent T, trimethylaminoacetohydrazide hydrochloride; trivial names of steroids, as defined in Tables I and II.