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A Lipophosphoprotein Complex in Hen Plasma Associated with Yolk Production

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The blood of the laying hen is characterized by a very high lipid content, especially of triglyceride (e.g. Lorenz, Entenman & Chaikoff, 1938; Walker, Taylor & Russell, 1951). It is also characterized by the presence of protein phosphorus ('serum vitellin') demonstrated by Laskowski (1935a) and Roepke & Hughes (1935), who observed its virtual absence from the serum of non-laying birds. The high lipid and phosphoprotein content of laying-hen plasma is apparently the result of high oestrogen production (see Lorenz, 1954, and Sturkie, 1954, for reviews).

Laskowski (1935b) obtained a phosphoprotein fraction as a precipitate by dilution of laying-hen plasma, and Roepke & Bushnell (1936) observed the precipitation of phosphoprotein on dialysis of hen serum. Both groups of workers, although apparently recognizing the presence of lipid in the precipitable material, made no study of it. More recently, McKinley, Oliver, Maw & Common (1953) and McKinley, Maw, Oliver & Common (1954) presented evidence for the occurrence of phosphoprotein in the material precipitated from the serum of pullets treated with oestrogens, on dilution.

This paper deals with the conditions of preparation of the precipitable lipophosphoprotein from laying-hen plasma, and observations on its composition and properties. In addition its behaviour during the pre- and post-laying periods has been studied. A preliminary account of some of this work has been given (McIndoe, 1957).

METHODS

Animals. Brown Leghorn hens (2 years old), fed on the Poultry Research Centre diet (Bolton, 1958), were used. They were kept in battery cages under artificial lighting. When it was desired to put the hens off lay the amount of light they received was reduced from 14 to 6 hr./day by 1 hr./day (0·5 hr. at either end). To bring them into production this procedure was reversed.

Preparation of precipitable lipophosphoprotein. Blood (usually 3–5 ml.) was taken by syringe from the wing vein and added to a small amount of sodium citrate (about 0·5 mg./ml. of blood). Plasma was diluted with 9 vol. of water, allowed to stand in the refrigerator for 1½–2 hr. and centrifuged at 3000 g for 20–30 min. at 2–5°. The supernatant fraction was retained. The precipitate was dissolved in water and reprecipitated by bringing the NaCl concentration to 0·015M. After standing at 2° for 15 min. the precipitate (PLP) was centrifuged as before for at least 40 min.

Extraction of lipids. Absolute ethanol (20 ml.) was added to the precipitate from 2 ml. of plasma, mixed and allowed to stand overnight. The protein was subsequently extracted with 20 ml. of ethanol–ether (3:1, v/v) three times and finally with ether. The extracts were combined, reduced to less than 10 ml. on a steam bath and evaporated to dryness in a vacuum desiccator over P_2O_5. The supernatant fraction was mixed with 4 vol. of ethanol and the lipids were extracted from the precipitated protein as described above. To remove water-soluble contaminants, water and ether were added to the combined aqueous ethanol–ether extract until an aqueous phase developed. The organic phase was evaporated to dryness as above. Before analysis the dried lipids were dissolved in dry $CHCl_3$.

Analysis of lipids. Total lipid was estimated gravimetrically. (Values for the supernatant fraction are approximate, owing to the relatively low lipid content.) Subsequently the phospholipid (P × 25) was determined by phosphorus analysis (Allen, 1940) and cholesterol by the Liebermann–Burchard reaction (Hawk, Oser & Summerson, 1947). The error in the latter estimation, due to the possible non-equivalent colour intensities of cholesterol and cholesterol esters, is likely to be small since only about 20% of hen-plasma cholesterol is esterified (Walker *et al.* 1951). In a few cases free and total cholesterol were determined by the above-mentioned colorimetric method after using the saponification and digitonin-precipitation procedures of Schoenheimer & Sperry (1934).

Protein. Samples of protein for the determination of N and P were dried at 110° for 18 hr. and allowed to cool in a

desiccator over P_2O_5 before weighing. Nitrogen was estimated by the micro-Kjeldahl method with a copper-selenium catalyst (Chibnall, Rees & Williams, 1943). Total phosphorus was estimated by the method of Allen (1940). Alkali-labile phosphate was determined by incubating the protein in 0·25N-NaOH soln. at 37° for 24 hr. (Plimmer & Bayliss, 1906), deproteinizing with trichloroacetic acid soln. (5%, w/v) and estimating inorganic phosphate by direct application of the method of Allen (1940); a trace of insoluble material which appeared on addition of molybdate was removed by centrifuging before addition of reducing reagent.

Ultracentrifugal analysis. PLP (0·4 g./100 ml.) in NaCl soln. of sp.gr. 1·006 and in KBr soln. of sp.gr. 1·063 was examined in the analytical ultracentrifuge (Spinco model E) at 52 640 rev./min. and at 18°.

RESULTS

When plasma from the laying hen is diluted with water an abundant precipitate forms almost immediately and this contains a large amount of lipid and also phosphoprotein. It flocculates within about an hour. Formation of a precipitate is not observed with plasma from non-laying birds, except those which will lay within 8 or 9 days or have laid their last egg within 3 days (i.e. birds which have developing ova in their ovary).

Precipitation is apparent at a dilution of 1 in 5 and is maximal at dilutions of 1 in 9 to >1 in 12. The material, after centrifuging, is well packed and has a yellow, translucent, waxy appearance, and can be dissolved in 0·15M NaCl soln. or in water. It precipitates immediately from aqueous solution on the addition of small amounts of 0·15M-NaCl. The material then is paler in colour, granular, does not flocculate and packs less well on centrifuging.

PLP, isolated by the procedure given in the Methods section, has a N/P ratio consistently close to 3·5 (Table 1). (All N/P ratios are ratios by weight.) A further three reprecipitations do not significantly alter this ratio, although the solubility of the preparation in water tends to decrease.

Solubility of precipitable lipophosphoprotein. PLP, at a final concentration of 0·2%, was added to NaCl solutions of differing strengths and the amount dissolving estimated by determining N and P. The results (Fig. 1) show that PLP is virtually insoluble in NaCl solutions between 0·01 and 0·04M and that the N/P ratio is close to 3·5 at all concentrations of NaCl. Prolonged dialysis (18 hr. at 2°) of PLP gives rise to material which also precipitates from dilute NaCl solutions but which has a N/P ratio of about 3, owing to protein (containing no P) remaining in solution.

The solubility of PLP (in 0·2M-sodium acetate–acetic acid buffer), based on determinations of N and P, is minimal at about pH 5·6 and is almost constant between pH 5·4 and 5·8. The N/P ratio of the precipitated material is near 3·5 at pH 5·0–6·0.

Composition of precipitable lipophosphoprotein. The more detailed analysis of PLP is presented in Table 2, in which results on the plasma concentration are included.

PLP consists of about 20% of protein, containing about 0·75% of P, and 80% of lipid of which 25% is phospholipid and 4% is cholesterol. (Two samples had 76 and 79% of the cholesterol in the free form.) The very high triglyceride content of laying-hen plasma (Walker *et al.* 1951) leads to the conclusion that the remaining PLP lipid is almost entirely triglyceride. The amount of PLP present in the plasma is high and very variable, even in the same hen. It is apparently a function of the number and size of rapidly growing follicles in the ovary (W. M. McIndoe, unpublished work).

After the investigation of PLP began it seemed worthwhile examining the remaining supernatant so that the proportion of whole plasma constituents which are carried by PLP could be assessed. Although the analysis of the supernatant is not entirely satisfactory for phospholipid and phosphoprotein (see Discussion), the results are presented in Table 3, PLP constituents being given as
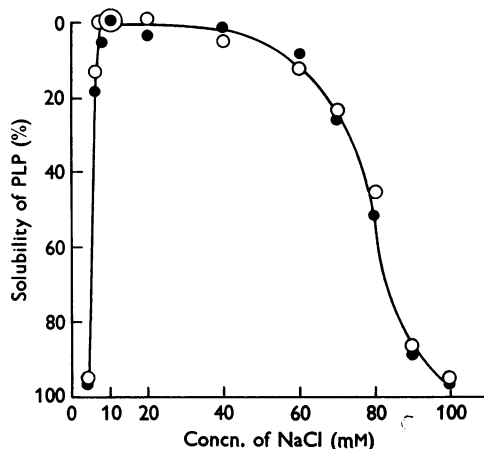

Fig. 1. Solubility of PLP in sodium chloride solutions. Concentration of PLP was 2 mg./ml. Results were obtained by analysis for phosphorus (●) and nitrogen (○).

Table 1. *Precipitable lipophosphoprotein nitrogen and phosphorus content of plasma*

	N (mg./100 ml. of plasma)	P (mg./100 ml. of plasma)	Ratio: N/P*
Mean (10 samples)	60·0	17·2	3·45
Range	49·3–70·0	14·6–20·1	3·26–3·72
S.D.	8·3	2·1	0·13

* Ratio by weight.

Table 2. *Concentration and percentage composition of plasma precipitable lipophosphoprotein*

| | PLP (g./100 ml. of plasma) | Protein | | Lipid | | |
		(N × 7) (% dry wt.)	Ratio: N/P*	Total (% dry wt.)	Phospholipid (% total lipid)	Cholesterol (% total lipid)
Mean (10 samples)	2·42	20·6	19·2	79·4	25·7	4·0
Range	1·36–4·28	19·4–22·5	16·7–22·7	77·5–80·6	24·1–27·3	3·7–4·3
S.D.	0·85	1·0	1·6	1·0	1·0	0·2

* Ratio by weight.

Table 3. *Percentage of whole plasma protein and lipid accounted for by precipitable lipophosphoprotein*

PLP constituents are expressed as percentage of the total in plasma.

| | Protein | | Lipid | | |
	N	P	Total	Phospholipid	Cholesterol
Mean	10·0	36·2	77·7	62·5	67·7
Range	6·4–13·6	22·5–47·8	65·7–92·0	45·1–79·9	49·6–85·3
S.D.	2·7	7·9	7·8	11·0	11·5

a percentage of whole plasma constituents. About 80% of plasma lipid is associated with PLP, which accounts for smaller proportions of plasma cholesterol and phospholipid. This indicates that almost all the plasma triglyceride is present in PLP. Only about 10% of plasma-protein N but approximately one-third of the protein P is contained in PLP.

Protein-bound phosphorus. After exhaustive extraction of lipid from PLP with ethanol–ether (3:1, v/v) at room temperature, the protein contains a considerable amount of phosphorus. Four large-scale preparations of PLP protein having a nitrogen content of 14·3, 14·5, 14·1 and 14·3% contained 0·79, 0·74, 0·71 and 0·72% of P respectively. (N/P ratios on a larger group are included in Table 2.) Most of this P is labile to alkali (0·25 N-NaOH at 37° for 16 hr.), 80–85% being liberated as inorganic phosphate and only about 2% remaining acid-insoluble. No more phosphate is released on further incubation (up to 60 hr.) and the liberation is virtually complete in 10 hr.

Similar results are obtained with the lipid-extracted protein in the supernatant obtained on precipitation of PLP. However, the proportion of protein P which is labile to alkali is lower and more variable (53, 50, 61 and 67% for four samples) and the proportion remaining acid-insoluble (6%) is greater than for PLP protein.

Stability of precipitable lipophosphoprotein to organic solvents. Examination of the stability of PLP lipid to organic solvents has not been extensive. Only trace amounts of lipid are immediately extracted from a PLP solution by gentle rotation (without breaking the interphase) with an equal volume of ether. About 50% of the lipid

(30% of the phospholipid) passes into the ether phase on standing for 4 hr. at 20°, although a latent period of somewhat less than 1 hr. is apparent before appreciable amounts are extracted. Approximately 75% (including 50% of the phospholipid) is removed if the ether contains 10% (v/v) of ethanol (see Macheboeuf & Sandor, 1932; Macheboeuf, 1953), and the latent period is probably reduced. If the PLP solution is vigorously shaken with ether almost 85% of the lipid (60% of the phospholipid) is extracted much more rapidly, though a short latent period is again observed. Similar results are obtained if the PLP solution is frozen below −25° in the presence of ether (McFarlane, 1942).

Heterogeneity of precipitable lipophosphoprotein. After most of the work presented was completed it became possible to examine PLP in an analytical ultracentrifuge. In a NaCl soln. of sp.gr. 1·006 two components separate, one of which floats (about 70% of the total calculating from the schlieren diagram: apparent S value −11) and one sediments (apparent S value 13). A qualitatively similar result is obtained in a potassium bromide solution of sp.gr. 1·063.

Precipitable lipophosphoprotein during pre- and post-ovulatory periods. PLP appears in the plasma of hens 7–8 days before the first ovulation (after they have been out of lay for at least 3 weeks) and increases in amount, reaching a maximum about 3 days before the first ovulation. Thereafter the plasma concentration decreases, reaching the relatively constant values for highly productive hens 2–5 days after the first ovulation (see Fig. 2 for a characteristic pattern). The composition of PLP changes slightly during this period (Table 4). The

initially higher protein and lower lipid content quickly revert to characteristic values as the concentration of PLP increases and the relatively high cholesterol content drops progressively.

Analysis of plasma before PLP is detectable and subsequently of the supernatant from the precipitation of PLP shows (Fig. 2) that the protein-bound P behaves similarly to PLP, although it appears in the plasma at least 24 hr. before the latter is evident. The lipids in the supernatant show a less marked but earlier increase, and the maximum occurs on the sixth or seventh day before ovulation. Supernatant protein N varies little during the pre-ovulatory period, though more often than not it is higher at the beginning than
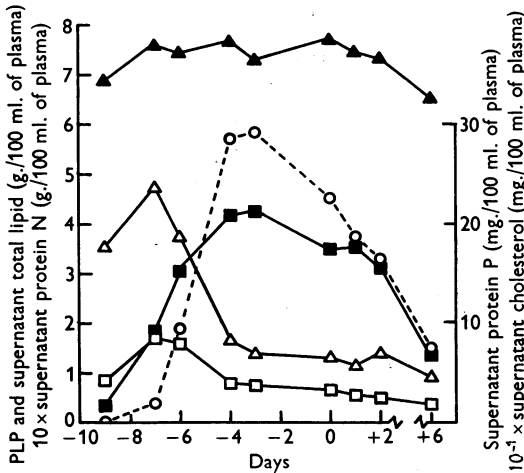

Fig. 2. Changes in the plasma concentration of PLP and of protein and lipid constituents of the supernatant (after removal of PLP) during the period when hens are coming into lay. Results are for one bird characteristic of a group of six. Abscissa gives time of sampling in days before (−) and after (+) the first ovulation (0). O, PLP. Supernatant constituents: ▲, protein nitrogen; ■, protein phosphorus; △, cholesterol; □, total lipid; (phospholipid follows a pattern similar to that for total lipid but at a slightly lower level).

when egg-laying is established and drops gradually throughout the period.

The investigation of PLP during the cessation of egg production has been less extensive. However, it is clear from Table 5 that PLP begins to disappear about the day of the last ovulation and is not present 4 days later. Instead, only a small amount of protein, containing trace amounts of lipid, is precipitable on dilution. This protein contains no P. In some individual birds this protein may persist for at least 14 days but disappears within 3 weeks. Coincident with the disappearance of PLP, protein P drops sharply almost to zero in the supernatant fraction of the plasma, supernatant total lipid and phospholipid decrease slightly and cholesterol increases from approximately 30 mg./100 ml. of plasma, before PLP decreases, to about 100 mg./100 ml. of plasma, when PLP has disappeared. The latter value is slightly lower than that for highly productive hens.

DISCUSSION

The constancy of composition of PLP prepared from different plasma samples and the results obtained on PLP precipitated at different sodium chloride concentrations and by isoelectric precipitation do not suggest that PLP is heterogeneous. However, examination in the analytical ultracentrifuge shows PLP to consist of two components, one of which is a light lipoprotein and the other a protein or dense lipoprotein. This fact should be kept in mind throughout the following discussion.

PLP contains a very high proportion of lipid (80 %). The apparent triglyceride content is very high and the cholesterol content is relatively low. Indeed the gross composition of the lipid is very similar to that of whole yolk. Also, it accounts for about 80 % of whole plasma lipid and apparently an even greater proportion of the plasma triglyceride. These facts, together with its behaviour when hens begin or stop laying, lead to the conclusion that PLP is carrying a very high proportion of the plasma lipid destined for inclusion in the

Table 4. *Composition of precipitable lipophosphoprotein during the period when hens are coming into lay*

Samples, taken from the same bird, are designated days before (−) or after (+) the first ovulation (0) and are those given in Fig. 2.

	Protein		Lipid		
Days	(N × 7) (% dry wt.)	Ratio: N/P*	Total (% dry wt.)	Phospholipid (% total lipid)	Cholesterol (% total lipid)
− 7	30·5	28·8	69·5	28·9	11·5
− 6	21·2	23·8	78·8	21·5	10·6
− 4	17·2	19·3	82·8	20·6	8·0
− 3	17·6	18·8	82·4	21·6	7·8
0	18·1	20·0	81·9	21·0	6·7
+ 1	18·7	19·2	81·3	22·9	6·5
+ 2	19·1	19·4	80·9	23·1	6·5

* Ratio by weight.

Table 5. *Composition and concentration of precipitable lipophosphoprotein when hens are going off lay*

Samples are designated days before (+) or after (−) the last ovulation (0).

| | | Precipitated material | | Composition of precipitated material | | | | | |
| | | | | Protein | | | Lipid | | |
Bird no.	Days	g./100 ml. of plasma	Ratio: N/P*	% dry wt. (N × 7)	mg. of N/ 100 ml. of plasma	Ratio: N/P*	Total (% dry wt.)	Phos- pholipid (% dry wt.)	Cholesterol (% dry wt.)
5	+5	1·63	3·82	19·6	45·6	24·9	80·4	22·5	3·4
4	+3	2·20	5·31	20·0	62·9	27·9	80·0	15·0	3·9
10	+3	1·87	5·70	21·4	57·2	20·0	78·6	13·2	2·1
11	+3	0·87	3·28	21·8	57·1	25·3	78·2	31·3	3·2
1	0	1·31	3·52	20·6	38·6	25·2	79·4	26·4	3·0
3	−2	0·71	4·62	23·8	24·1	27·0	76·2	23·7	3·1
1	−3	0·21	22·5	50·0	16·2	50·6	50·0	5·0	— §
3	−4	<0·10	44·3	90·0	8·8	— ‡	10·0	5·0	—
8	−4	<0·10	—†	>90·0	12·0	—	Trace	<1·0	—
6	−5	<0·10	—	>90·0	10·2	—	Trace	<1·0	—
7	−5	<0·10	—	>90·0	4·4	—	Trace	<1·0	—
9	−5	<0·10	—	>90·0	9·4	—	Trace	<1·0	—
12	−5	<0·10	—	>90·0	11·3	—	Trace	<1·0	—
2	−7	<0·10	—	>90·0	10·0	—	Trace	<1·0	—

* Ratio by weight.
‡ Phosphorus not detectable.
† Only a trace of phospholipid phosphorus was present.
§ Not estimated.

yolk. Recently a fraction with properties similar to those of PLP has been isolated from yolk (W. M. McIndoe, unpublished work) and is being investigated. However, according to Schmidt, Bessman, Hickey & Thannhauser (1956), only a small proportion of yolk triglyceride appears to be associated with phosphoprotein.

Recently, electrophoresis and the ultracentrifuge have been used to examine the plasma lipoproteins of the domestic fowl. Electrophoresis has demonstrated the occurrence of a lipoprotein component in the serum of the laying hen which is not obvious in immature birds (Moore, 1945, 1948). It apparently contains a high proportion of lipid but the protein accounts for only a small proportion of total serum protein. PLP may be related to this lipoprotein. There is also an increase in lipoprotein of laying hens in the β-globulin region and a decrease in the α_1-globulin area (Moore, 1948). This picture has been confirmed in general by Brandt, Clegg & Andrews (1951) and Clegg & Hein (1953) in hens and by Clegg et al. (1951) and Clegg & Hein (1953) in cocks treated with oestrogens. The paper-electrophoresis study of serum from laying hens and pullets treated with oestrogens, by McKinley et al. (1953, 1954), also confirms these findings in part.

Schjeide (1954), using the ultracentrifuge, has observed the occurrence of a large amount of lipoprotein in laying-hen serum corresponding to β-lipoprotein and lighter components. Hillyard, Entenman & Chaikoff (1956), using the same technique, have examined the serum of cocks treated with oestrogens and find large amounts of

a fraction having a high triglyceride content and floating at a lower specific gravity than does β-lipoprotein. It is not present in normal cock serum. This fraction is probably related to the low-density component of PLP. These workers also observed an increased β-lipoprotein fraction and a decreased α_1-lipoprotein fraction in the serum of cocks treated with oestrogens. The report of Schjeide & Urist (1956) indicates a similar situation.

The lipid composition of the plasma of laying hens compared with that of non-laying birds is a reflexion of the changes in the lipoprotein fractions. The decrease in α_1-lipoprotein, which is relatively rich in cholesterol and especially phospholipid (Hillyard et al. 1956), would tend to offset the effect of the appearance of PLP and the increase in the β-lipoprotein fraction, at least for cholesterol and phospholipid. (Note the similar plasma cholesterol of layers and non-layers.)

The protein moiety of PLP, containing about 0·74% of phosphorus, is similar to that which Laskowski (1935b) prepared from the original precipitate obtained by ten times dilution of serum at pH 5·0–5·5 and which contained 0·53% of phosphorus (the lower pH may have caused precipitation of material in addition to PLP). By salt-fractionation of the original precipitate Laskowski (1935b) obtained a component with a protein phosphorus content of 0·9%. A fraction similar to the latter was obtained by Roepke & Bushnell (1936) from material precipitated from laying-hen serum on dialysis. In agreement with the latter workers the material precipitated by dilution contains less than half the total plasma-protein phosphorus.

The present methods of estimating phospho-protein phosphorus are not satisfactory (Ågren, de Verdier & Glomset, 1954). Methods based on the lability to alkali of phosphoprotein phosphorus (Plimmer & Bayliss, 1906) may give low results owing to incomplete liberation of phosphate; phosphoserine and phosphoserine peptides have been detected in alkaline hydrolysates of proteins (Hutchison et al. 1956; Phillips, 1956; Glomset, 1957; Hawthorne, 1957). Diesterified protein phosphate may be more stable than the monoester form. Also the amino acid environment of the phosphoseryl residues may influence the proportion of inorganic phosphate liberated, as is indicated by the work of Plimmer & Bayliss (1906) and of Rimington (1941).

In the present work it has been presumed that all the protein-bound phosphorus is phosphoprotein phosphorus. This may be true of PLP but the protein of the plasma supernatant fraction is probably contaminated with phospholipid in a form non-extractable by ethanol (McIndoe, 1958). Thus in this fraction estimates of phosphoprotein phosphorus may be high and phospholipid estimates correspondingly low. The protein phosphorus of PLP behaves towards alkali in a way very similar to that found by Plimmer & Scott (1908) for 'vitellin' phosphorus. It should be pointed out that no phosphorylated amino acid has been isolated from the proteins of laying-hen plasma, though phosphoserine has been isolated from yolk proteins (Lipmann & Levene, 1932; Levene & Schormuller, 1933).

In the hen follicles begin to grow rapidly 7–9 days before ovulation (Romanoff, 1931, 1943; Warren & Conrad, 1939; Entenman, Ruben, Pearlman, Lorenz & Chaikoff, 1938; Lorenz, Pearlman & Chaikoff, 1943), and the appearance of PLP 7 or 8 days before ovulation coincides with this. Also, PLP and phosphoprotein disappear rapidly from the plasma when egg production ceases, in agreement with Hosoda, Kaneko, Mogi & Abe (1955), who have shown the disappearance of 'serum vitellin' by immunological methods within 5 days of the last egg being laid.

The total protein content of the plasma is apparently similar in laying and non-laying hens, though the range of values is large (D. J. Bell, unpublished work; Sturkie & Newman, 1951; see also Sturkie, 1954). However, during a short immediately pre-ovulatory period the plasma protein level is higher than it is in the truly non-laying state or when egg-laying is established. This is due to the presence of a large amount of PLP at this time (Fig. 2). This is in confirmation of Greenberg, Larson, Pearson & Burmester (1936) and of Vanstone, Maw & Common (1955), who worked with fowls, and of McDonald & Riddle (1945),

working with pigeons. Other workers have made similar observations on the high pre-ovulatory level of lipids in the plasma of doves and pigeons (Riddle & Burns, 1927; Riddle, 1942), and in the blood of hens (Lorenz et al. 1938; Chaikoff, Lorenz & Entenman, 1941), and of protein phosphorus in pigeon plasma (McDonald & Riddle, 1945). These high values may be partially attributed to the sub-maximal size of the growing follicles which are 'absorbing' material into the yolk. However, on the day of the first ovulation PLP and phosphoprotein are still higher than when high egg production is established. The very high protein phosphorus content of the supernatant (from PLP preparation) during the pre-ovulatory period without an increase in protein nitrogen may be explained if, as in egg yolk, most of the phosphoprotein phosphorus is present as phosvitin (Mecham & Olcott, 1949), which contains 10 % of phosphorus.

Changes in the composition of PLP occur when hens are coming into and going out of production, i.e. at times when their physiological state is altering rapidly. This may be due to an alteration in the proportions of the components of PLP separable in the ultracentrifuge or in the composition of these components. The effect of nutritional or hormonal changes in altering lipoprotein composition has been noted by Macheboeuf (1929), Macheboeuf & Rebeyrotte (1949) and by Hillyard et al. (1956).

PLP should provide very suitable material for the investigation of the chemistry and biosynthesis of lipoprotein (of high triglyceride content) and phosphoprotein. It should also supply the means of elucidating the problem of yolk formation, i.e. the selective 'absorption' of high-molecular-weight substances by the hen ovary against a concentration gradient.

SUMMARY

1. A lipophosphoprotein complex (PLP) of constant composition can be precipitated from laying-hen plasma by dilution with water, and a method for its preparation and estimation has been developed. It is not detectable in the plasma of non-laying hens or of cocks.

2. The concentration of PLP is about 2 g./ 100 ml. of plasma and is very variable. It consists of about 20 % of protein and 80 % of lipid (of which 25 % is phospholipid and 4 % is cholesterol, largely free). The remaining lipid is probably largely or wholly triglyceride. The protein contains approximately 0·75 % of phosphorus (80 % of which is very labile to alkali, indicating that it is phosphoprotein).

3. PLP accounts for a large but variable proportion of whole plasma lipids; i.e. total lipid, about 80 %; phospholipid and cholesterol, approxi-

mately 65 %. It also accounts for about 35 % of the plasma protein phosphorus and 10 % of the protein nitrogen.

4. PLP appears in hen plasma about 7 days before the first ovulation and disappears within a few days when hens go off lay. The plasma concentration and composition of PLP during these periods have been examined. Details of changes in the lipid and protein nitrogen and phosphorus of the supernatant obtained from the preparation of PLP are also given.

5. Preliminary ultracentrifugal analysis of PLP has shown it to consist of two components. The major one floats in a sodium chloride solution of specific gravity 1·006, with an apparent $S - 11$, and the other sediments (apparent S 13).

6. It is possible that PLP transports most of the plasma lipid and almost half the plasma phosphoprotein included in egg yolk.

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