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Role of avidin and other biotin-binding proteins in the deposition and distribution of biotin in chicken eggs

Discovery of a new biotin-binding protein

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In addition to the previously characterized egg-yolk biotin-binding protein (BBP-I), we have discovered another BBP (BBP-II) in the plasma and yolk from laying hens. BBP-I is stable to 65 °C, whereas BBP-II is stable to 45 °C. Both proteins are normally saturated with biotin and together they account for most, if not all, of the biotin in hen plasma and yolk, except in hens fed excessive amounts of biotin (> 1 mg of biotin/kg of feed). The maximal production of BBP-I is attained at lower levels of dietary biotin (~ 50 μg/kg) than for BBP-II (~ 250 μg/kg); however, the maximal production of BBP-II is severalfold greater than for BBP-I. Consequently, as dietary biotin increases, the ratio of BBP-II to BBP-I increases and becomes constant at dietary intakes of biotin above 250 μg/kg. The observation that the amounts of these proteins are limited by biotin in the normal dietary range (< 250 μg/kg) suggests that biotin is required for the synthesis, secretion or stability of these proteins. Although both plasma vitamin–protein complexes are transported to the oocyte and concentrated in the yolk, BBP-II is transferred more efficiently. Thus biotin deposition in the yolk is a function of the amounts and relative concentrations of the two proteins. Dietary biotin above 250 μg/kg exceeds the transport capacity of BBP-I and BBP-II in the plasma; however, unbound biotin does not accumulate. Rather it is efficiently scavenged by avidin in the oviduct and transferred to the egg albumen. Only when avidin becomes saturated at high dietary intake does free or weakly bound biotin accumulate in plasma and yolk. The synthesis of avidin is independent of dietary biotin. Small amounts of BBPs with the heat-stability of avidin or BBP-I respectively are present in the plasma of adult males or immature chickens. BBP-II, the major BBP in the plasma and yolk of laying hens, was not detected in the plasma of non-laying chickens.

INTRODUCTION

In 1927 Boas showed that there was a nutritional factor in egg yolk and other foods that was inactivated by a heat-labile component of egg albumen. Biotin, the nutritional factor, was first isolated and characterized from duck egg yolk (Kögel & Tönnis, 1936). Avidin, the antivitamin, was purified from egg albumen (Pennington *et al.*, 1942) and shown to be an extraordinarily stable tetrameric protein whose affinity for biotin may well be the strongest non-covalent interaction between a protein and a small molecule (Green, 1975). György & Rose (1942) observed that biotin in egg yolk became dialysable on heating, but it was more than 30 years before a biotin-binding protein (BBP) was identified and purified from egg yolk (White *et al.*, 1976; Meslar *et al.*, 1978; Murty & Adiga, 1984). Though similar in size and quaternary structure, BBP is distinct from avidin by a number of criteria. This protein is also present in the plasma of laying hens (Mandella *et al.*, 1978) and is presumed to be deposited in the ovarian follicle along with other egg-yolk proteins (White, 1985).

The original assay for yolk BBP was based on the equilibrium exchange of endogenous bound biotin and exogenous [^{14}C]biotin at 65 °C (Meslar & White, 1979). Although this assay is quite satisfactory for partially purified BBP, it was technically difficult to perform on yolk extracts, and the limits of detection were approached with plasma samples. Furthermore, the calculations for this assay included corrections for isotope dilution which assumed that the protein was initially saturated with unlabelled biotin and that no additional endogenous biotin was present. Recently a new and much more sensitive assay based on the exchange binding of [^{3}H]biotin has been developed (White & McGahan, 1986; H. B. White, T. McGahan & M. A. Letavic, unpublished work). The graphical analysis of this assay yields the amount of endogenous ligand (Lotter *et al.*, 1982). Although there are still some technical problems with this assay that cause underestimates of the amount of binding proteins, it is now possible to analyse BBP in samples not accessible with the previous assay.

In the process of applying this new assay to yolk and plasma samples from lying hens that had been fed diets differing in their biotin content, we discovered that the endogenous biotin of content of yolk and plasma exceeded the BBP binding sites by severalfold. This was unexpected, because this excess biotin should have been dialysable. The paradox was resolved by the discovery of a second BBP (BBP-II) that binds most, if not all, of the biotin not bound by the previously recognized BBP hereafter designated 'BBP-I'.

Abbreviation used: BBP, biotin-binding protein.

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Table 1. Composition of experimental diets

Vitamin/mineral supplements provided (per kg of diet): A: copper, 3.6 mg; iodide, 0.4 mg; iron, 80 mg; magnesium, 300 mg; manganese, 100 mg; zinc, 50 mg; retinol, 600 μg; cholecalciferol, 15 μg; α-tocopherol, 17 mg; menadione, 1.3 mg; riboflavin, 4 mg; nicotinic acid, 28 mg; panthothenic acid, 10 mg; B: as for A, plus cyanocobalamin, 25 μg; folic acid, 0.5 mg; pyridoxine, 4 mg; thiamin, 2 mg; C: as for A, plus biotin, 100 μg; choline chloride, 0.5 g.

	Composition (g/kg)		
Ingredient	Laying diet	Biotin-deficient laying diet	Chick diet
Maize	—	—	50
Wheat	750	553	596
Starch	–	80	30
Herring meal	60	—	145
Meat and bone meal	30	—	—
Soybean meal	25	—	105
Casein (low-vitamin)	—	100	—
Gelatin	—	48	—
Egg albumen (spray-dried)	—	36	—
Isolated soy protein	20	—	40
Vegetable oil	—	30	—
Cellulose	—	36	—
Limestone flour	65	76	13
Dicalcium phosphate	22	30	12
Salt	3	4	3
DL-Methionine	—	2	—
Vitamin/mineral supplements			
A	5	—	—
B	—	5	—
C	—	—	3

In the present paper we document the presence of BBP-II and show that it is unstable under the conditions used for the assay of BBP-I. Furthermore we show that neither the deposition of biotin in yolk nor the distribution of biotin between yolk and albumen is a simple function of dietary biotin or plasma biotin concentration. The patterns can be explained by the differential synthesis and differential transport of BBP-I and BBP-II.

MATERIALS AND METHODS

Experimental design

ISA Brown hens (96 in all, housed in individual battery cages) were maintained on a standard laying diet for several weeks until a high rate of egg production was established. The diet, the composition of which is given in Table 1, was based on wheat and contained a relatively low amount of available biotin (~ 28 mg/kg), but was nevertheless thought to be aequate in all nutrients for maximal egg production (Whitehead, 1980). Seven groups of 12 hens each were fed the standard diet supplemented with 0, 100, 250, 500, 1000, 2000 and 4000 μg biotin/kg of feed. An eighth group was fed a biotin-deficient diet (Table 1). This diet contained hen's-egg albumen as a source of avidin and was thought to be virtually devoid of available biotin. Daily egg productions and weekly feed consumptions were recorded. Plasma, yolk and albumen were collected weekly and analysed for biotin and biotin-binding proteins.

In a second experiment, newly hatched chicks were sexed and then fed a diet (Table 1) containing about 160 mg of available biotin/kg. Blood samples were taken from several chicks and pooled at various ages and analysed for BBPs.

Sample preparation

Plasma, yolk and albumen samples were obtained exactly as described by White *et al.* (1986) for similar studies on riboflavin, except that the 4-fold dilutions of egg yolk were made with 50 mM-sodium acetate, pH 5.5, containing 50 mM-NaCl. Samples were stored frozen at −20 °C until assayed for BBP. On the day of analysis, plasma samples were usually diluted 10-fold, yolk samples an additional 10–50-fold and albumen samples 200-fold with the above buffer. Samples for biotin analysis were not diluted before freezing.

Assays for BBP-I

BBP-I was assayed by a radioligand-exchange procedure analogous to that described for assaying riboflavin-binding protein (Lotter *et al.*, 1982). A series of tubes containing 0.1 μCi of D-[8,9-^{3}H(n)]biotin (lot 2169-146, 35.0 Ci/mmol; New England Nuclear Corp., Boston, MA, U.S.A.) and 20–240 μl of diluted plasma or yolk in the above sodium acetate buffer (total volume 1.0 ml) was incubated at 65 °C for 40 min to equilibrate free and BBP-I-bound biotin. These conditions denature BBP-II. The cooled incubation mixtures were quantitatively transferred to small phosphocellulose columns [0.25 ml bed volume in polypropylene pipette tips (Sarsted, no. 91-787)]. Uncomplexed biotin was eluted with two 1.0 ml buffer washes. BBP-I-bound biotin was then eluted directly into scintillation vials by washing the columns with 4 × 0.25 ml of the sodium acetate buffer

containing 2 M-NaCl. A portion (10 ml) of scintillation-counting fluid (Opti Phase 'X', Amersham International) was added and the amount of radioactivity determined in a liquid-scintillation counter. Non-specific binding was determined in the presence of 500-fold excess of unlabelled biotin, and an avidin solution was used to determine total bindable radioactivity. Data were plotted according to the following linear equation (White & McGahan, 1986) (the slope and intercepts were determined by linear-regression analysis):

$$\frac{{}^*L_T}{{}^*L_B} = \frac{1}{[P_T]} \cdot \frac{{}^*L_T \cdot F}{V} + \frac{L_T}{P_T}$$

If ${}^*L_T/{}^*L_B$, the ratio of total to bound radioactive ligand is plotted as a function of ${}^*L_T \cdot F/V$, where F and V are the dilution factor and volume of the diluted sample used in the assay; the y-intercept, L_T/P_T, is the ratio of endogenous biotin to biotin-binding sites, and the reciprocal of the slope, $[P_T]$, is the concentration of binding protein in the undiluted sample.

Assays for BBP-II

An assay that directly measures BBP-II in the presence of BBP-I has not been developed. Two procedures have been used to estimate BBP-II activity. The first method was exactly like that described for BBP-I except that the incubation temperature was 45 °C instead of 65 °C. The choice of temperatures was based on the thermal-stability profiles presented in Figs. 5 and 6 (below). The difference between the results from the 45 °C and 65 °C assays was attributed to BBP-II, but this approximation is in error to the extent that BBP-I does not achieve equilibrium exchange at 45 °C. Alternatively BBP-II activity has been estimated from the BBP-I assays by assuming that BBP-II is saturated with biotin and all endogenous biotin in excess of BBP-I was bound to BBP-II. Although both of these assays are qualitatively reliable, the values obtained are systematically low compared with the values expected from bioassay for biotin.

Assays for avidin

Avidin was measured in albumen or plasma exactly as for BBP-I, except the incubation was at 85 °C, a temperature that destroys BBP-I and BBP-II. Unoccupied biotin-binding sites were determined at room temperature, where exchange does not occur.

Biotin analyses

Undiluted yolk, plasma and albumen samples were frozen and sent with feed samples to F. Hoffmann–LaRoche, Basel, Switzerland, where they were assayed for biotin by using *Lactobacillus plantarum* as described by Frigg & Brubacher (1976).

RESULTS

Biotin content of diets

The diets were formulated to contain 30, 100, 250, 500, 1000, 2000, and 4000 μg of biotin/kg. The analyses of these diets showed respectively 93, 165, 332, 622, 1054, 1831 and 3540 μg total biotin/kg. The data plotted in the Figures correspond to available biotin which is about 65 μg/kg less than the measured values. This correction is justified by the fact that over 95% of the biotin in wheat is not available (Frigg, 1976; Whitehead *et al.*, 1982).

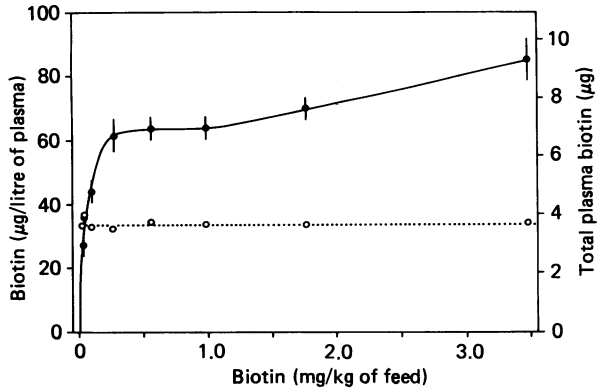

Fig. 1. Effect of dietary biotin on the concentration of biotin the plasma of laying hens

Open circles (○) are the control values for the treatment groups immediately before the experimental diets were begun. The dotted line represents the average of these control values (33.9 ± 1.99 μg/l). Closed circles (●) represent the average ± S.D. for samples taken after 1, 2, and 5 weeks on the experimental diets. The right-hand scale is based on a calculated blood volume of 109 ml.

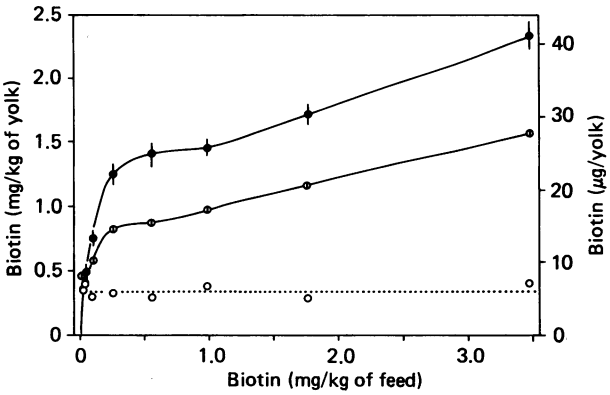

Fig. 2. Effect of dietary biotin on the concentration of biotin in chicken egg yolk

Open circles (○) represent control values for yolk samples from the treatment group immediately before the experimental diets were begun. The dotted line represents the average of these control values (343.1 ± 48.3 μg/kg). The divided circles (⦶) represent the average for duplicate yolk samples after the birds had been 1 week on the experimental diets. Closed circles (●) represent the mean ± S.D. for duplicate samples taken after the birds had been 2 and 5 weeks on the experimental diet. The right-hand scale is based on an average yolk weight of 17.6 g.

Biotin content of plasma, yolk and albumen as a function of dietary biotin

Figs. 1–3 show respectively the biotin levels in plasma, yolk and albumen before and after 1, 2 and 5 weeks on the experimental diets. Steady-state levels were achieved within 1 week in plasma and albumen and by 2 weeks in yolk. With respect to dietary biotin, the accumulation of biotin in plasma, yolk and albumen can be considered in three phases. In the normal range of dietary biotin (< 250 μg/kg) there is a very strong relationship

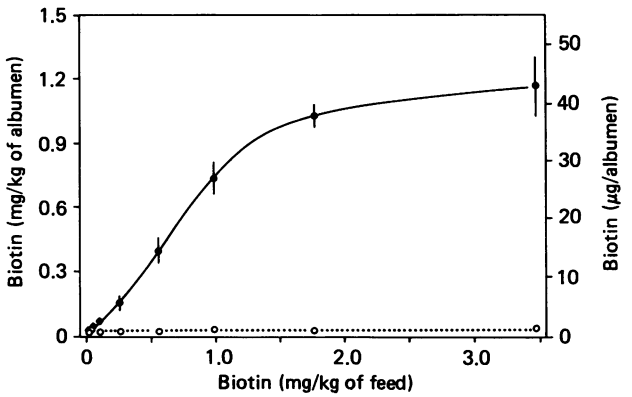

Fig. 3. Effect of dietary biotin on the amount of biotin in chicken egg albumen

Open circles (○) represent the control values for the treatment groups immediately before the experimental diets were begun. The dotted line represents the average of these control values (26.4±6.7 μg/kg). Closed circles (●) represent the average±S.D. for duplicate samples taken after the birds had been 1, 2 and 5 weeks on the experimental diets. The right-hand scale is based on a recovered albumen weight of 36.6 g/egg.

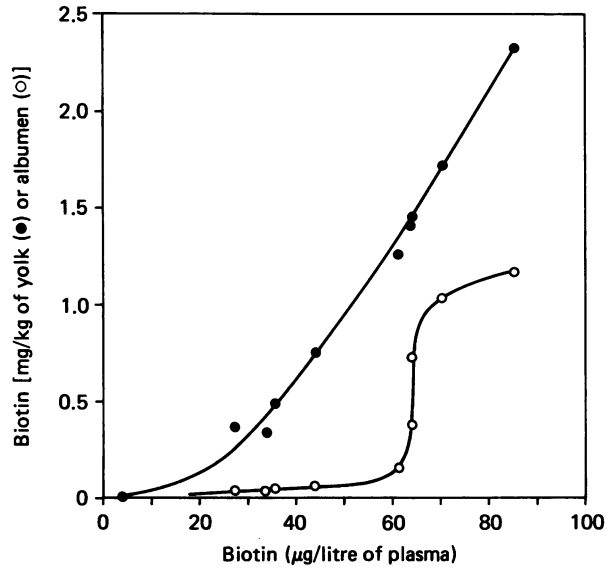

Fig. 4. Concentration of biotin in the yolk (●) and albumen (○) of chicken eggs as a function of the concentration of biotin in the plasma of the laying hen

Each point represents the average of four to six biotin determinations made on plasma, yolk and albumen samples. Note the vertical scale is 25 times that of the horizontal scale.

between dietary biotin and the biotin content of plasma and yolk, whereas the biotin content of albumen is rather low. Between 250 and 1000 μg/kg there is a plateau in the biotin contents of plasma and yolk and a large increase in the biotin content of albumen. Above 1000 μg of biotin/kg there is a further gradual increase in biotin in all three compartments. Considering the range from 30 to 3475 μg/kg, an increase of over 100-fold in dietary biotin, there is a 2.5-fold increase in plasma biotin, a 6.8-fold increase in yolk biotin and a 44.5-fold increase in albumen biotin. Hens fed the biotin-deficient diets for 6 weeks continued to lay eggs. The plasma and yolk concentrations of biotin from these birds were 4.05 μg/l and 13.0 μg/kg respectively. The biotin content of albumen was below the detection limits of the assay. These responses are broadly in agreement with the observations of Frigg *et al.* (1984).

Biotin content of yolk and albumen as a function of plasma biotin

The plasma distributes absorbed dietary biotin to the various tissues of the body, and thus biotin in the plasma is an intermediate between ingested biotin and biotin deposited in the yolk and albumen of eggs. Fig. 4 shows that biotin deposition in yolk and albumen is not a simple function of plasma biotin concentration. Although biotin is concentrated in yolk relative to plasma over the entire range of experimental conditions, the efficiency of transfer is greatly reduced when plasma biotin concentrations are below 20 μg/l. The yolk-to-plasma concentration ratio in this region is less than 10:1, whereas the incremental increase above this region is near 35:1. The relationship between biotin in albumen and plasma shows an even more dramatic discontinuity. Below 64 μg/l, very little biotin is deposited in albumen. A slight increase in plasma biotin above this level results in very large increases in the deposition of biotin in albumen.

BBP-I and BBP-II content of plasma and yolk

Fig. 5 shows that hens transferred from a diet containing 30 μg of biotin/kg of feed to one containing 987 μg/kg increase the production of a heat-labile BBP appearing in both the plasma and yolk. The data in Fig. 5 are not corrected for isotope dilution. If such a correction were made, there would be little difference in the total biotin binding above 60 °C, whereas difference in biotin-binding near 45 °C would be accentuated.

The kinetics of biotin exchange at 45 and 65 °C in the same yolk samples are shown in Fig. 6. Again, the increase in the amount of heat-labile BBP-II is evident in the samples from hens fed high-biotin diets. There is very little difference in the amount of BBP-I in these two samples.

Similar analyses conducted at 45 and 65 °C on yolk samples from hens fed a wide range of dietary biotin (Fig. 7) show that maximal production of BBP-I occurs on diets containing 50 μg or more of biotin per kg of feed, whereas maximal production of BBP-II occurs with higher levels of dietary biotin (> 250 μg/kg). The slightly lower amounts of BBP-I at higher dietary biotin levels are not considered signficant at this time because the large amounts of endogenous biotin render the BBP-I assay less accurate in this region.

Fig. 8 shows that there is an 8–10-fold excess of endogenous biotin over BBP-I in yolk from hens fed very high levels of biotin. The mean±S.D. of this ratio for 20 assays performed on yolk samples from pre-experimental control hens maintained on 30 μg of biotin/kg of diet is 1.64±0.20. If one assumes that the excess biotin in the samples is due to BBP-II bound biotin, the ratio of BBP-II to BBP-I increases from 0.64 at 30 μg of biotin/kg to 5 at 500 μg/kg. The biotin content of yolk is included in Fig. 8 as a reference to show that the data obtained by microbiological assay are qualitatively similar to those

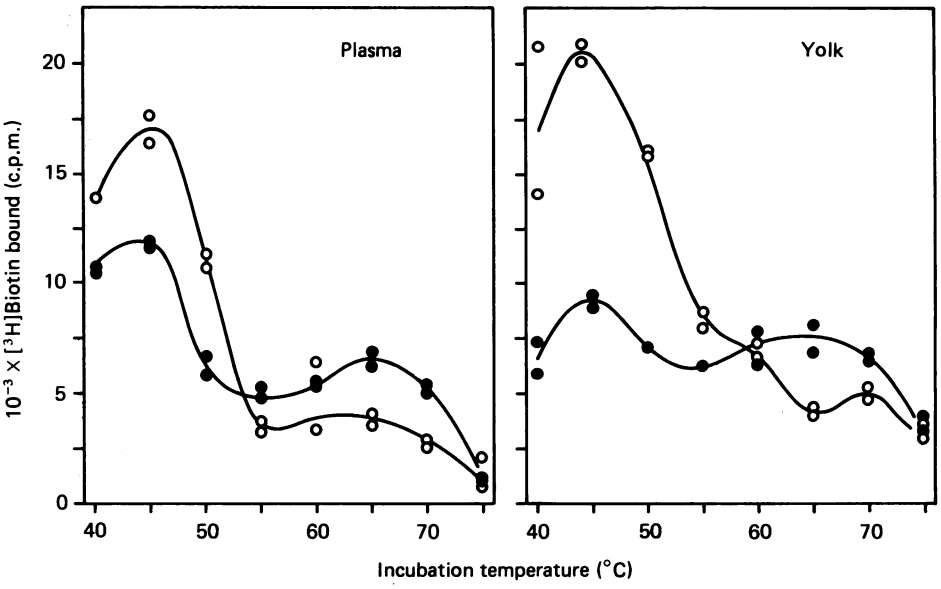

Fig. 5. Concentration of a heat-labile BBP increases in the plasma and egg yolk from hens fed diets with high concentrations of biotin

The equivalent of 10 μl of plasma and 1 μl of yolk from hens fed 28 μg of biotin/kg of feed (●) were incubated for 30 min in the presence of [^{3}H]biotin, and the bound [^{3}H]biotin determined. Similar samples from the same birds were analysed after they had been maintained for 3 weeks on the standard diet supplemented to 987 μg of biotin/kg (○). The amount of endogenous biotin, and thus the isotope dilution, in these samples is greater than those from the samples from hens on the low-biotin diet.

obtained by isotope-exchange assays of the binding protein. Assays at 45 °C show that BBP-II is saturated with biotin even in yolk samples obtained from hens fed a biotin-deficient diet for 5 weeks.

Content and fractional saturation of avidin in albumen

Table 2 shows that the concentration of avidin in albumen is unaffected by dietary biotin. The fractional saturation is strongly dependent on dietary biotin. Avidin is over 90% saturated with biotin when dietary biotin exceeds about 1500 μg/kg.

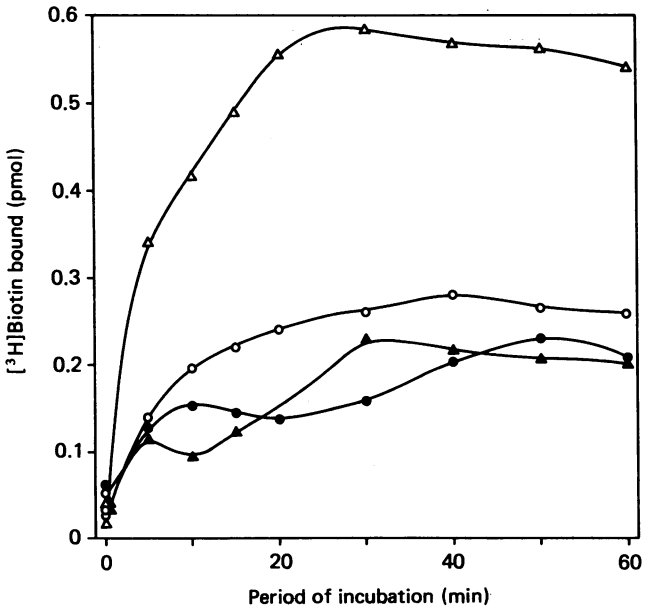

Fig. 6. Kinetics of biotin exchange at 45 °C (○, △) and 65 °C (●, ▲) show the accumulation of a heat-labile biotin-binding protein in egg yolks from hens fed a diet with 30 μg of biotin/kg of feed (○, ●) and then fed a diet with 1000 μg/kg for 3 weeks (△, ▲)

BBPs in the plasma of immature and adult chickens of both sexes

As noted in the Methods and materials section, the amounts of the various binding proteins cannot be measured with high accuracy when they are present in mixtures. Table 3 presents the analysis of BBP-I, BBP-II and avidin in the plasma of immature and adult male and female chickens. The identification is based on heat-stability. BBP-I is present in the plasma of immature chickens of both sexes at concentrations about one-tenth that found in laying hens. BBP-II was detected only in the plasma of laying hens. Avidin was present in

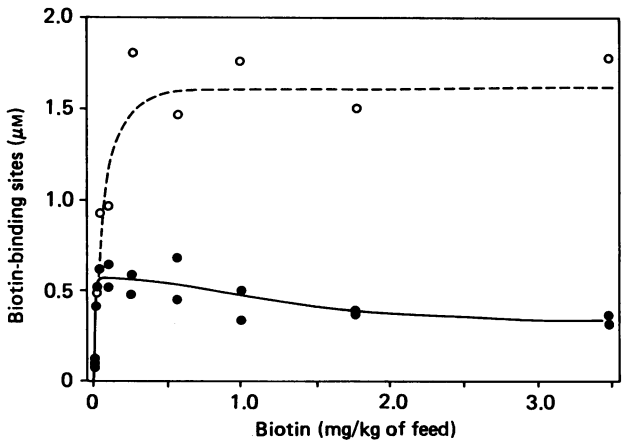

Fig. 7. Concentration of biotin-binding sites in chicken egg yolk determined by radioligand exchange at 45 °C (○) and 65 °C (●) as a function of dietary biotin

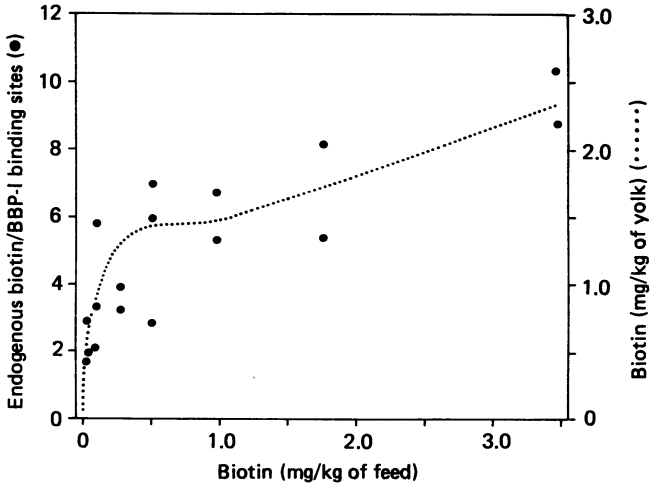

Fig. 8. Estimation of the ratio of endogenous biotin to BBP-1 binding sites in yolk as a function of dietary biotin

The dotted reference line is the microbiologically determined endogenous biotin content of the same samples from Fig. 2 scaled to coincide with the data points.

Table 3. Estimation of the amounts of various biotin-binding proteins in the plasma of immature and adult chickens

Values are expressed as the concentration of biotin-binding sites as determined by radioligand exchange assays for 40 min at 45 °C for BBP-II, 65 °C for BBP-I and 85 °C for avidin.

		Concn. (nM)		
Age	Sex	BBP-II	BBP-I	Avidin
1 day	Male	< 0.6	~ 1.8	< 1.0
	Female	< 0.5	~ 2.3	< 1.5
2 weeks	Male	< 1.0	~ 2.6	< 1.3
3 weeks	Female	< 0.5	~ 1.5	< 0.5
6 weeks	Female	< 0.8	~ 3.0	< 0.8
8 weeks	Male	< 1.0	~ 4.2	< 0.9
9 weeks	Female	< 0.8	~ 2.7	< 0.7
11 weeks	Male	< 0.6	~ 2.3	< 1.2
Adult	Female	40–120	25–40	n.d.*
	Male	~ 0	< 2.9	10.5

* n.d., not determined.

significant amounts only in the plasma of adult males. In all samples, the amount of biotin equals or exceeds the available binding sites.

DISCUSSION

Discovery of BBP-II

As originally designed, there were two objectives in the present study. The first was to determine if biotin transport to the yolk was limited by BBP. The other was to determine if apo-BBP could be generated *in vivo* and be transported to yolk. At the time, only one BBP was known from yolk and it was stable at 65 °C. Our initial results at 65 °C (Fig. 8) with a new assay based on [^{3}H]biotin exchange produced the expected saturation profile, but unexpectedly revealed severalfold more biotin in yolk than biotin-binding sites. This paradox was resolved when a second, more abundant but heat-labile, BBP (BBP-II) was discovered. The discovery of BBP-II seems to be dependent in part on the new assay, which uses [^{3}H]biotin of high specific radioactivity and permits a 100-fold higher dilution of samples (L. Bush & H. B. White III, unpublished work). [^{14}C]Biotin-based analyses of egg yolk which should have contained BBP-II show no heat-labile component below 70 °C (White *et al.*, 1976; Kulomaa *et al.*, 1981). Furthermore, the earlier assay procedure had not been developed so that the presence of excess endogenous biotin could be detected. Although quantification of the amounts of BBP-I and BBP-II in mixtures is imprecise, the general patterns that have emerged are clear, as discussed below.

Relationship of plasma biotin to yolk biotin

The deposition of biotin in yolk is not the linear or saturable function of biotin in the plasma that would be expected for a simple receptor-mediated or diffusional process. Rather, biotin at low concentrations in plasma is transferred to yolk less efficiently than it is at higher concentrations (Fig. 4). This pattern can be qualitatively explained by the presence of two BBPs whose production is differentially dependent on dietary biotin and whose efficiencies of transfer to yolk are different.

The plasma concentration of BBP-I is rather constant in laying hens unless dietary biotin is severely restricted, in which case the concentration of BBP-I is lower. The fact that BBP-I remains saturated with biotin even in biotin-deficient hens (Fig. 8) indicates that the synthesis, secretion or stability of this protein is dependent on biotin. The pattern for the production of BBP-II is similar, except that a greater response is observed and the response saturates at higher dietary biotin. Thus over the

Table 2. Biotin-binding capacity and biotin saturation of avidin in albumen from hens fed different amounts of biotin

Dietary biotin (μg/kg)	28	100	257	557	987	1766	3475
Biotin-binding capacity* (μg/kg of albumen)							
Pretreatment	5.37	7.52	5.34	5.78	5.68	5.25	5.15
Week 1	—	6.49	7.60	5.49	6.15	4.64	4.39
Week 2	—	7.23	5.64	5.53	6.60	4.74	4.88
Week 5	4.66	5.17	4.80	3.86	5.11	4.17	4.31
Biotin saturation (%) (average for weeks 1 and 2)	6.3	22.3	54.3	70.1	81.5	94.4	97.2

* Values reported have been corrected for losses due to avidin binding to surfaces at the high dilutions used in the [^{3}H]biotin-based assay.

normal dietary biotin range the production of BBP-II relative to BBP-I increases from less than 1 at 30 μg of biotin/kg of feed to perhaps greater than 5 at 250 μg/kg. The concentration ratio of BBP-II to BBP-I in yolk is greater than in plasma, indicating that BBP-II is deposited more efficiently in the yolk than is BBP-I.

We conclude that normal biotin deposition in egg is dependent on, and stoichiometric with, BBPs, as previously asserted (White, 1985). However, at very high dietary biotin levels there is increased biotin deposition in yolk in the absence of increased production of BBP. This additional biotin behaves as free biotin in our assay. The unexpectedly high efficiency with which this 'free' biotin is transferred from plasma to yolk suggests that it may in fact be bound weakly to a second site on BBP-II or be associated with lipids (Trager, 1948).

Dietary biotin absorbed in excess of that necessary to saturate BBP-I and BBP-II at maximal production should appear as free biotin; however, as indicated above, 'free' biotin is not detected in plasma or yolk until dietary biotin is considerably increased. This excess biotin in the plasma is scavenged very efficiently by avidin in the oviduct. Only when avidin becomes saturated are there significant increases in free biotin in plasma and yolk (Figs. 1 and 2).

Regulation of the production of BBP-I and BBP-II

The synthesis of both BBP-I and BBP-II is stimulated during egg laying. This implies that the gene (or genes) for these two proteins is (are) induced by oestrogen, as has been suggested by Murty & Adiga (1985). The observation that the production of BBP-I and BBP-II was also dependent on biotin availability was unexpected. Thus both sex hormones and a specific ligand regulate the production of both proteins. The mechanism of the biotin effect is not known. Although transcriptional regulation is possible, a translational regulation can be envisaged in which ligand binding to the nascent protein must occur before termination or secretion. A less efficient biotin-dependent mechanism could depend on the instability of the apoprotein.

Mechanism of biotin deposition in the oocyte

Ultrastructural studies show that yolk deposition occurs via a very active clathrin-mediated endocytosis (Perry *et al.*, 1978, 1984; Griffin *et al.*, 1984). The deposition of specific proteins such as vitellogenin and low-density lipoproteins has been shown to be receptor-mediated (Woods & Roth, 1984; Krummins & Roth, 1981). Despite the presence of these receptors, the concentration of these proteins in yolk is only about six times greater than in plasma. Cholecalciferol is concentrated by this same factor and is thought to be deposited as a complex of its binding protein and the phosvitin moiety of vitellogenin (Fraser & Emtage, 1976). Similarly riboflavin is concentrated 6-fold in yolk (White *et al.*, 1976), yet no receptor has been detected for its binding protein (Benore-Parsons, 1986). Other plasma-derived yolk proteins, such as immunoglobulins, serum albumin and transferrin, are not concentrated in yolk (Schjeide *et al.*, 1976), even though receptors for immunoglobins have been reported.

Biotin is concentrated by more than 20-fold in yolk relative to plasma. This ratio varies from about 3 when chickens are fed biotin-deficient diets to almost 30 when diets of high protein content are fed. In comparison with other yolk-to-plasma concentration ratios, these values are quite high and suggest that a specific receptor-mediated transport system exists for BBP-II and perhaps BBP-I as well.

Transfer of biotin-binding proteins from yolk to chick plasma

Several yolk proteins are transferred to the plasma of the embryo. For instance, the immunity of the hen is transferred to the chick via immunoglobulins deposited in yolk (Loecken & Roth, 1983). Similarly, maternal serum transferrin, in pigeons at least, appears in the chick plasma (Frelinger, 1971). The presence of BBP-I in the plasma of newly hatched chicks (Table 3) suggests that there may be transfer of BBP-I from the yolk. Although this possibility cannot be ruled out, the fact that the concentration of BBP-I remains fairly constant during the rapid growth of a chick implies that most, if not all, BBP-I in older chicks at least is synthesized by the chick and not derived from the yolk. The apparent absence of BBP-II in chick plasma precludes significant yolk-to-plasma transfer.

Relationship of BBP-I to BBP-II

Although it is clear that yolk BBPs are distinct from egg-white avidin (Meslar *et al.*, 1978; Murthy & Adiga, 1984), the structural and genetic relationships between BBP-I and BBP-II are yet to be determined. The fact that their concentrations show different dependences on dietary biotin, that their distribution differs between hen and chick plasma, and that their distributions between soluble and particulate fractions of diluted egg yolk differ (results not shown) suggest that two different gene products are present. However, they could be differently modified products of the same gene. Furthermore, there is the possibility that both are tetrameric isoproteins which can generate hybrid intermediate forms that associate with a receptor or other proteins with different affinities.

Whatever the structural and genetic relationships are between BBP-I and BBP-II, there seems to be a functional differentiation of the two proteins. BBP-I is present in the plasma of immature chickens. Despite its increased concentration in the plasma of laying hens relative to chick plasma, it is transferred to yolk less efficiently than is BBP-II. BBP-II, on the other hand, is detected only in the plasma of laying hens and is normally present at higher concentrations than BBP-I. These patterns suggest that BBP-I has primarily a maintenance function and secondarily it serves to transport biotin to yolk. The primary and perhaps sole function of BBP-II seems to be the transport of biotin to the yolk.

Function of avidin

Avidin has been viewed as one of the many antimicrobial proteins of egg albumen (Tranter & Board, 1982). This view is further supported by the induction of avidin synthesis at the site of tissue injury in chickens (Elo & Korpela, 1984). The absence of significant amounts of biotin in egg albumen suggest that avidin has little role in the biotin nutrition of the embryo. Our results are consistent with a non-nutritive role for avidin and show that biotin deposition in albumen occurs only when chickens are fed on diets that have biotin contents well in excess of those of natural foods. We also observed

a saturated BBP with the stability of avidin in the plasma of adult males (Table 1), confirming the earlier observations of Elo *et al* (1979). Perhaps avidin does have a role in biotin metabolism in cockerels.

Comparison of biotin and riboflavin transport to the chicken egg yolk

Parallel studies on the binding-protein-mediated deposition of riboflavin to the oocyte (White *et al.*, 1986) show a different and much simpler pattern than described here for biotin. Riboflavin availability does not regulate the production of riboflavin-binding protein. Apoprotin is produced and deposited in yolk. From this sample of two vitamins, it is clear that generalizations about the mechanism of protein-mediated vitamin transport to the oocyte may be hard to find.

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