Avian vitellogenin consists of two major species designated VTG I and VTG II. Rooster hepatocytes were employed to identify intracellular forms of the vitellogenins and to characterize biosynthetic intermediates of VTG I and VTG II. After labeling with ¹³C-serine, intracellular vitellogenin radioactivity was seen in mature VTG I and VTG II but was primarily found in two species, pVTG I and pVTG II, which showed greater mobilities in sodium dodecyl sulfate-polyacrylamide gels. The pVTG species were identified as vitellogenins by reaction with antibodies against plasma VTG II and against the mixture of VTG I and VTG II. Immunological and peptide mapping procedures were used to relate pVTG I and pVTG II to secreted VTG I and VTG II, respectively. Pulse-labeling and pulse-chase experiments showed that the pVTG species are precursors to the secreted vitellogenins and are thus discrete intermediates in the biosynthesis of the vitellogenins. Additional labeling experiments showed that the pVTG species are glycosylated but not phosphorylated. The stages of vitellogenin biosynthesis may be ordered as follows: polypeptide synthesis → glycosylation → phosphorylation → secretion. The presence of only small quantities of the phosphorylated vitellogenins intracellularly indicates that when phosphorylation is completed, the vitellogenins are rapidly secreted from the hepatocyte.

The differences in the electrophoretic mobilities of the pVTG and VTG species suggested that sodium dodecyl sulfate-polyacrylamide gel electrophoresis does not accurately estimate the molecular weights of the heavily phosphorylated vitellogenins. This was confirmed directly by showing that the mobility of plasma vitellogenin increased upon dephosphorylation. An independent estimate of vitellogenin molecular weight was made by gel chromatography in 7 M guanidine-HCl. With this method, the molecular weights of the pVTG and VTG species were indistinguishable and in agreement with the molecular weight of the pVTG species as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These analyses indicate that the vitellogenin polypeptide has $M_\text{r} = 180,000$. This value is 60,000–70,000 less than commonly estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The implications of this lower molecular weight are discussed in relation to vitellogenin structure and the egg yolk polypeptides which may derive from each vitellogenin.

Vitellogenin is the major yolk precursor protein synthesized by and secreted from the livers of oviparous vertebrates in response to estrogenic stimulation (1, 2). After uptake into the developing oocyte, vitellogenin is cleaved into a complex group of yolk proteins which act as nutrient sources for embryonic development. The enormous increases in hepatic vitellogenin synthesis and vitellogenin mRNA following hormone treatment have focused considerable attention on avian and amphibian vitellogenins as models for estrogen-regulated gene expression (1–3). Recent studies indicate that vitellogenin is not a unique protein, but appears to be a small family of similar proteins encoded by several genes (4, 5). At the protein level, heterogeneity has been noted in amphibian vitellogenin (6) and two avian vitellogenins, VTG I and VTG II, have been purified from plasma of estrogen-stimulated roosters (7). Comparisons of amino acid compositions, peptide maps, and immunological properties indicate substantial differences in the structures of the two avian vitellogenins. Both vitellogenins are unusually large proteins with apparent $M_\text{r} = 260,000$ and 246,000 for VTG I and VTG II, respectively, as judged by SDS-polyacrylamide gel electrophoresis. Each vitellogenin also contains 2% phosphorus (7) and is glycosylated. The vitellogenins are thus large complex proteins which must undergo extensive post-translational modification in the hepatocyte. Surprisingly, little is known about vitellogenin biosynthesis or the influence of the post-translational modifications upon the physical and biological properties of these proteins. Such studies have been difficult because of the complexity of these proteins as well as the inability to analyze specific vitellogenins as discrete molecular species (1, 6, 8).

We report here experiments which identify and characterize two discrete intracellular vitellogenins which are precursors to avian VTG I and VTG II. The intracellular vitellogenins are found almost exclusively as nonphosphorylated polypeptides, but only the fully phosphorylated vitellogenins are secreted. These and other results permit us to order the stages of vitellogenin biosynthesis as follows: polypeptide synthesis → glycosylation → phosphorylation → secretion. The presence of only small quantities of the phosphorylated vitellogenins intracellularly suggests that, when phosphorylation is completed, the vitellogenins are rapidly secreted from the hepatocyte.

* This research was supported by Grant AM 18171 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: VTG, vitellogenin; SDS, sodium dodecyl sulfate; PMSF, phenylmethylsulfonyl fluoride; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl.

2 S.-Y. Wang, and D. L. Williams, manuscript in preparation.
toocyte. Additionally, we report the surprising result that the avian vitellogenin polypeptides are 60,000–70,000 daltons smaller than generally estimated by SDS-polyacrylamide gel electrophoresis. This result has important implications for the vitellogenin structure as well as for the elucidation of which egg yolk polypeptides are derived from each vitellogenin.

EXPERIMENTAL PROCEDURES

Hormone Treatment, Hepatocyte Preparation, and Radiolabeling—White Leghorn roosters (0.6–1 kg, SPAFAS, Norwich, CN) received an intramuscular injection of diethylstilbestrol (50 mg/kg) in propylene glycol 3 days prior to killing. Laying hens received no treatment. Hepatocytes were prepared by the method previously described for modification of the two-step collagenase perfusion method developed by Seglen (10). Hepatoctyes exhibit viabilities greater than 95% as judged by trypan blue exclusion and decline in viability by no more than 10% during 4 h of suspension culture (9). Radiolabeled amino acid incorporation into protein is linear for at least 4 h (9). Hepatoctyes (5 × 10⁶/ml) were incubated at 40°C in modified minimal essential medium (9) containing 5% chicken serum and one of the following: [³H]leucine (100 µCi/ml, [4,5-³H]leucine, 12 Ci/mmol, New England Nuclear), [³H]serine (26 µCi/ml, [3-³H]serine, 19 Ci/mmol, Amersham), or [³H]glucosamine (19.6 µCi/ml, [6-³H]glucosamine, 15 Ci/mmol, New England Nuclear). Cells were removed by centrifugation for 3 min at 60 × g and washed once in iced balanced salt solution (9). Medium was centrifuged again for 1 min at 10,000 × g to ensure complete removal of cells. Sample buffer of electrophoretic system A (11) was added to the medium; the sample was boiled for 3 min and analyzed by electrophoresis as indicated below or stored at −70°C until analysis. The washed hepatocytes were solubilized by boiling in electrophoresis sample buffer and analyzed by electrophoresis. For immunological analyses, cells were homogenized at 0–4°C in 0.02 M sodium phosphate, pH 7.4, 0.15 M NaCl, 0.005 M ethylenediaminetetraacetic acid, 1% Triton X-100, 100 µg/ml PMSF, and debris was removed by centrifugation for 5 min at 10,000 × g. In several experiments, the influence of proteolysis was tested by including the following inhibitors singly or in combination in homogenization buffer or electrophoresis sample buffer in addition to PMSF: aprotinin (10 units/ml), pepstatin A (1 µg/ml), disopropylfluorophosphate (5 mM), benzamidine (5 mM), and 2,3-dimercaptopropanol (10 mM).

For in vitro tissue incubation, a liver slice (10 mg) was chopped into four pieces and incubated for 1 h at 40°C in 0.05 ml of Krebs-Ringer bicarbonate buffer (12) containing 30 or 250 µCi of [³H]leucine or [³H]serine under an atmosphere of 95% O₂/5% CO₂. The tissue was washed with 2 ml of iced Krebs-Ringer buffer, homogenized in 0.15 ml of electrophoresis sample buffer containing 100 µg/ml PMSF, boiled for 3 min, and analyzed by electrophoresis. In vivo labeling was carried out by injecting [³H]leucine ([5 mCi/ml) intraperitoneally in 15–20 g chickens. For in vitro tissue incubation, liver slices were homogenized and analyzed by electrophoresis sample buffer boiled, and analyzed by electrophoresis. Immunological Analysis—Rabbit antibody against VTG II (anti-VTG II) has been described (7). Antibody against the unresolved mixture of VTG I and VTG II (anti-VTG M) was prepared in the same fashion (7). Direct immunoprecipitations of hepatocyte extracts or incubation medium were carried out with unlabeled antigen as carrier at 50–75% of equivalence (7). Washed immunoprecipitates were dissolved in electrophoresis sample buffer, boiled, and analyzed by SDS-polyacrylamide gel electrophoresis.

Electrophoresis—Partial Proteolysis—Electrophoresis was carried out in a system described (System A Ref. 11) with SDS-polyacrylamide slab gels using the buffer system of Laemmli (13). The running gel contained either 5, 7.5, or 10% acrylamide as indicated. Radioactive proteins were visualized by fluorography (14). In several experiments, [³H]leucine incorporation into cellular and secreted vitellogenin was determined by cutting the appropriate bands from the dried gel. The gel sections were combusted, and radioactivity was recovered for scintillation counting as described (9). For partial proteolysis mapping (15), liver slices were labeled with [³H]leucine or [³H]serine as noted above and run on a SDS-5% polyacrylamide gel. Diallylated vitellogenins were added to the sample to facilitate identification of the vitellogenin region of the gel, which was excised and lyophilized. The dried gel was rehydrated in the minimum necessary volume of protease digestion buffer (7) containing 0.2 mg/ml V8 protease from Staphylococcus aureus (Miles). After incubation for 2 h at 37°C, the gel was boiled for 3 min, loaded onto a second slab gel at 90° to the first dimension, and run into an SDS-10% polyacrylamide gel. Lyophilization of the gel segment after first dimension electrophoresis markedly improved the reproducibility of the digestion kinetics in the gel, presumably because the protease enters the gel quickly during rehydration.

Dephosphorylation of Plasma Vitellogenins—Vitellogenin was dephosphorylated with bacterial alkaline phosphatase as described for egg yolk vitellogenin (BAPC, 27.7 units/mg, Worthington) at 37°C in 0.025 M 2-(N-morpholino)ethanesulfonic acid, pH 5.5, containing 100 µg/ml PMSF. The reaction was stopped by boiling or by the addition of trichloroacetic acid to a concentration of 5%. In the latter case, samples were centrifuged for 5 min at 10,000 × g and the supernatant was measured (16) in the supernatant to assess the extent of dephosphorylation. The initial phosphorus content was determined as phosphorus released by alkaline hydrolysis from the trichloroacetic acid-insoluble pellet of samples taken at time 0. Dephosphorylation experiments were carried out with vitellogenin preparations enriched in VTG I and preparations enriched in VTG II. When the dephosphorylated vitellogenins were run on SDS-5% polyacrylamide gels, [³H]serine-labeled hepatocyte extracts were run in adjacent gel lanes to determine the mobilities of each VTG and the corresponding non-phosphorylated precursor.

Molecular Weight Estimation by Gel Chromatography—Protein samples were S-carboxymethylated (17) and chromatographed on a Sepharose CL-4B column (55 × 0.9 cm) with 7 M guanidine-HCl as eluting solvent. Purified VTG II, a mixture of VTG I and VTG II, and protein standards were dissolved in or dialyzed into 7 M guanidine-HCl, 0.1 M tris(hydroxymethyl)aminomethane, pH 8.6, 0.06 M ethylenediaminetetraacetic acid, 0.19 M β-mercaptoethanol and held at room temperature for 4 h. Iodoacetic acid was subsequently added, and after 30 min, the sample was applied to the column and eluted at 1.6 ml/h with 7 M guanidine-HCl. Column fractions were monitored for radioactivity, and elution positions were monitored in terms of the weight of eluting solvent (19). Excluded and included elution weights were determined with dextran blue and [³H]leucine, respectively. A calibration curve was constructed as recommended by Ackers (19) and Fish et al. (20) with the following protein standards: human apolipoprotein B (M₀ = 250,000), myosin heavy chain (M₀ = 200,000), β-galactosidase (M₀ = 116,200), bovine serum albumin (M₀ = 68,000), ovalbumin (M₀ = 43,000), deoxyribonuclease (M₀ = 31,000), and ribonuclease A (M₀ = 14,000). Human low density lipoprotein (1.006–1.06 g/cm³) was prepared from fresh plasma by standard procedures (21) except that 100 µg/ml PMSF was included in all solutions. Apolipoprotein B was isolated and delipidated (22) by two cycles of chromatography on Sepharose 6B in 0.05 M sodium phosphate, pH 7, 1% SDS, 100 µg/ml PMSF (11) and extensively dialyzed against 7 M guanidine-HCl prior to S-carboxymethylisation and chromatography. For the chromatographic analysis of cellular VTG and pVTG species, [³H]serine-labeled liver tissue was homogenized in iced 10% trichloroacetic acid; the insoluble material was collected by centrifugation, washed three times with iced sterile water, dissolved in 7 M guanidine-HCl and S-carboxymethylated as above. After chromatography, individual fractions were dialyzed extensively against 0.02 M tris(hydroxymethyl)aminomethane, pH 8.6, 100 µg/ml PMSF at 4°C, lyophilized, and analyzed by SDS-5% polyacrylamide gel electrophoresis.

RESULTS

Occurrence of Putative VTG Precursors—Vitellogenin accounts for as much as 10% of liver protein synthesis in estrogen-stimulated roosters as judged by [³H]leucine incorporation (23–26). This feature as well as the high serine content of plasma vitellogenin (7, 8) suggested that [³H]serine should selectively label newly synthesized VTG I and VTG II. The results of Fig. 1 indicate that this is the case. This figure shows the electrophoretic profile of newly synthesized proteins after estrogen-stimulated hepatocytes were labeled with [³H]serine for 1 h. The SDS-5% polyacrylamide gel was run in the time necessary for the tracking dye to reach the gel bottom in order to resolve proteins in the M₀ = 100,000–400,000 range. As anticipated, the proteins secreted by hepatocytes (lane 2) show two bands which co-migrate with plasma VTG I and

These reaction conditions were suggested by S. Wiley and R. Wallace.

3 These reaction conditions were suggested by S. Wiley and R. Wallace.
Biosynthesis of the Vitellogenins

Fig. 1. Electrophoretic profiles of cellular and secreted 
H-labeled proteins. Hepatocytes were incubated with [H]serine for 1 h, washed, and solubilized by boiling in SDS sample buffer. Solubilized hepatocytes (lane 1) and incubation medium containing secreted proteins (lane 2) were run on an SDS-5% polyacrylamide slab gel. Radioactive proteins were visualized by fluorography. Plasma VTG I and VTG II were run in an adjacent gel lane. Protein standards for molecular weight estimation were as described (11). Apo B, chicken apolipoprotein B.

VTG II and are present in approximately the same proportions as the two plasma vitellogenins (7). The secreted vitellogenins were seen only with hepatocytes from estrogen-stimulated roosters and laying hens but not with hepatocytes from untreated roosters. In addition, these proteins were further identified as VTG I and VTG II by reaction with anti-VTG M, an antiserum raised against plasma VTG I and VTG I1 (data not shown).

In contrast to the profile of secreted proteins (Fig. 1, lane 2), newly synthesized cellular proteins (Fig. 1, lane 1) show two prominent bands designated pVTG I and pVTG II with apparent \( M_r \) = 200,000 and 190,000 respectively, in comparison to 260,000 and 246,000 for secreted VTG I and VTG II (7). As seen here and in experiments described below, pVTG I and pVTG II are in similar proportions to secreted VTG I and VTG II. The cellular pVTG bands were observed only with hepatocytes from estrogen-stimulated roosters and laying hens, but not with hepatocytes from untreated roosters. In addition, the pVTG bands were seen when estrogen-stimulated liver slices were labeled \textit{in vitro} with either [H]serine or [H]leucine and when liver proteins were labeled \textit{in vivo} with [H]leucine (data not shown). The occurrence of the pVTG species, therefore, is dependent upon estrogen but is independent of the \textit{in vitro} labeling conditions and the preparation of hepatocytes. These results suggest that the pVTG species were intracellular vitellogenins. Note that the electrophoretic mobility of apolipoprotein B of very low density lipoprotein (11, 27-29) is the same in cellular (Fig. 1, lane 1) and secreted (Fig. 1, lane 2) proteins. This result indicates the absence of significant proteolytic activity in the cell extract and suggests that the greater electrophoretic mobilities of pVTG I and pVTG II as compared to the secreted vitellogenins did not result from proteolysis. Additional experiments to eliminate the role of proteolysis are described below.

Identification of pVTG as Nonphosphorylated Vitellogenins—The pVTG were further identified as vitellogenins by virtue of their reactivity with anti-VTG M, an antibody raised against the mixture of VTG I and VTG II purified from plasma (7). As shown in Fig. 2 (lane 3), pVTG I and pVTG II were selectively precipitated by anti-VTG M while apolipoprotein B was not (compare the immunoprecipitate in lane 3 with the profile of total [H]serine-labeled hepatocyte proteins in lane 1). Note that a faint VTG II band can be seen in the immunoprecipitate (lane 3) and a VTG I band can also be detected with a longer fluorographic exposure (data not shown). This result indicates that small quantities of cellular VTG I and VTG II are present, but the great majority of the newly synthesized vitellogenin occurs as the pVTG species. Densitometric tracings of either [H]serine or [H]leucine-labeled hepatocyte proteins show that pVTG I and pVTG II account for 94-98% of the cellular vitellogenin after labeling periods of 1, 2, or 3 h (data not shown).

Post-translational modifications of the cellular vitellogenins were examined by labeling hepatocytes with [H]glucosamine or \(^{32}\)P. After labeling with [H]glucosamine, cellular proteins show significant incorporation into pVTG I, pVTG II, and VTG II (Fig. 3, lane 1), each of which is precipitated by anti-VTG M (Fig. 3, lane 2). Longer exposure of the fluorographs shows labeling of cellular VTG I as well (data not shown). The [H]glucosamine-labeled vitellogenins (Fig. 3, lane 2) have the same or very similar electrophoretic mobilities compared to the corresponding pVTG and VTG species labeled with [H]serine (Fig. 3, lane 3). This result indicates that both the pVTG and VTG species are at least partially glycosylated. In studies to be reported elsewhere, we have observed that intracellular vitellogenins labeled with [H]serine in the pres-
FIG. 3. Cellular pVTG I and pVTG II are labeled with [3H]glucosamine. Hepatocytes labeled for 1 h with [3H]glucosamine (lane 1) or [3H]serine (lane 3) were washed, solubilized in SDS sample buffer, and run on an SDS-5% polyacrylamide slab gel. Another sample of [3H]glucosamine-labeled hepatocytes was homogenized in buffer for immunoprecipitation, and the anti-VTG M immunoprecipitate of this extract was run in lane 2. Plasma VTG I and VTG II were run in an adjacent gel lane. Radioactive proteins were visualized by fluorography.

Biosynthesis of the Vitellogenins

FIG. 4. Immunological identification of cellular and secreted 32P-vitellogenins. Hepatocytes were labeled with 32P, washed, and homogenized in buffer for immunoprecipitation as described under "Experimental Procedures." Cell extract (lane 1), the anti-VTG M immunoprecipitate of cell extract (lane 2), the anti-VTG II immunoprecipitate of cell extract (lane 3), total secreted proteins (lane 4), the anti-VTG M immunoprecipitate of secreted proteins (lane 5), and the anti-VTG II immunoprecipitate of secreted proteins (lane 6) were run on an SDS-5% polyacrylamide slab gel. Plasma VTG I and VTG II were run in an adjacent gel lane. Radioactive proteins were visualized by autoradiography.

contain both carbohydrate and phosphorus.

Specific Identification of pVTG I and pVTG II—The similarity in the proportions of newly synthesized pVTG I and pVTG II (Figs. 1 and 2) as compared to VTG I and VTG II in secreted proteins (Figs. 1 and 2) or in plasma (7) suggested that pVTG I and pVTG II might be specific precursors to VTG I and VTG II, respectively. These relationships were evaluated via immunological and peptide mapping procedures. First, cellular proteins labeled with [3H]serine were reacted with either anti-VTG M or antiserum raised against plasma VTG II (anti-VTG II) (7) to determine whether anti-VTG II showed selectivity for pVTG II. The immunoprecipitates were electrophoresed on an SDS-5% polyacrylamide gel and the pVTG II/pVTG I ratio was determined by densitometry of the fluorograph. The ratio with anti-VTG II was 7 while the ratio with anti-VTG M was 3. pVTG II is thus selectively precipitated by antiserum specific to plasma VTG II (7). The fact that some pVTG I is precipitated by anti-VTG II may indicate a minor degree of cross-reactivity not previously detected (7) or may reflect co-precipitation of pVTG I that is present in heterodimers with pVTG II. The latter possibility is likely in view of the recent finding that dimers of VTG I and VTG II are present in rooster plasma (7) A similar selectivity was also shown for cellular and secreted 32P-VTG species. As shown in Fig. 4, anti-VTG M precipitated both

4 G. AB, personal communication.
cellular (lane 2) and secreted (lane 5) \(^{32}\text{P}\)VTG I and \(^{32}\text{P}\)VTG II while anti-VTG II (lanes 3 and 6) selectively precipitated \(^{32}\text{P}\)VTG II. The selective reactivity of cellular pVTG II and VTG II with anti-VTG II relates these species specifically to plasma VTG II. The poor reactivity of pVTG I with anti-VTG II as compared to anti-VTG M indicates immunological dissimilarity between pVTG I and pVTG II, suggesting indirectly that pVTG I is related to VTG I.

Partial proteolysis mapping (15) was carried out to relate pVTG I and pVTG II to cellular and secreted VTG I and VTG II. After in vitro labeling with \(^{3}H\)leucine or \(^{3}H\)serine, tissue slices and medium were combined and solubilized directly in SDS sample buffer, and proteins were resolved on an SDS-5% polyacrylamide gel. The gel region including the pVTG and VTG bands was excised and treated with V8 protease (see "Experimental Procedures"), and the resultant peptides were analyzed in a second dimension SDS-10% polyacrylamide gel. As shown in Fig. 5, \(^{3}H\)leucine-labeled peptides 1-11 are present in digests of both pVTG II and VTG II, indicating a high degree of structural similarity between these species. Although the labeling intensity of VTG I is much less, peptides 12-15 are evident in digests of both pVTG I and VTG I. Other peptide mapping experiments have also shown homology in the pVTG I and VTG I digests and dissimilarity with peptides produced from pVTG II and VTG II (data not shown). pVTG I and pVTG II thus appear to be distinct polypeptides which show considerable structural relatedness to secreted VTG I and VTG II, respectively.

When processed in the oocyte, vitellogenin gives rise to two groups of proteins the amino acid compositions of which reflect the nonuniform distribution of particular amino acids within the vitellogenin polypeptide. Vitellogenin regions corresponding to the yolk phosvitins are very rich in phosphoserine and poor in leucine (1, 8, 32) while regions corresponding to yolk lipovitellins contain both serine and leucine in slight predominance (8, 33). V8 protease cleavage products of vitellogenins or vitellogenin precursors should also reflect the nonuniform distribution of these amino acids. Cleavage of plasma VTG II, in fact, yields a peptide distribution such that most of the phosphopeptides run in the \(M_r = 80,000-100,000\) range and most of the non-phosphorylated peptides run at lower molecular weights on SDS-10% polyacrylamide gels (7). Region A in Fig. 5 is the molecular weight range which should contain many of the serine-rich, leucine-poor phosphopeptides of VTG II. Note that the region A peptides of pVTG II do not have corresponding peptides of identical mobility in the VTG I digest. In contrast, the region A VTG II peptides appear to run somewhat slower than the

![Figure 5](https://via.placeholder.com/150)

**Fig. 5.** Partial proteolysis mapping of pVTG and VTG species. After incubation for 1 h with \(^{3}H\)leucine, liver slices and medium were solubilized in SDS-sample buffer, mixed with dansylated VTG I and VTG II, and run on an SDS-5% polyacrylamide slab gel. The gel region containing the vitellogenins was located under ultraviolet light, excised, and lyophilized. After digestion with V8 protease as described under "Experimental Procedures," the digest was run into an SDS-10% polyacrylamide slab gel at 90° to the direction of the first electrophoresis. Radioactive peptides were visualized by fluorography. Peptide designations are described in the text.

![Figure 6](https://via.placeholder.com/150)

**Fig. 6.** Comparison by partial proteolysis of pVTG II and VTG II after labeling with \(^{3}H\)serine and \(^{3}H\)leucine. After liver slices were labeled with either \(^{3}H\)leucine or \(^{3}H\)serine, first dimension SDS-5% polyacrylamide slab gels and V8 protease digestions were carried out as described under "Experimental Procedures." Gel segments containing digests of \(^{3}H\)serine (A) or \(^{3}H\)leucine (B)-labeled vitellogenins were run on the same SDS-10% polyacrylamide slab gel. The intensity of the fluorographic exposure permits comparison of pVTG II and VTG II peptides but not pVTG I and VTG I. The differences in first dimension separations evident in A and B are due to the fact that the first dimensions were run on different gels. Peptide designations are described in the text.
region A peptides of pVTG II. Since the vitellogenin phosphates reduce the electrophoretic mobility of vitellogenin (vide infra), slower mobilities of the VTG II region A peptides may be anticipated if these peptides, in fact, arise from the serine-rich vitellogenin region. To determine whether this is the case, V8 protease maps of pVTG I1 and VTG I1 were compared after hepatocytes were labeled with either \([\text{H}]\)serine (Fig. 6A) or \([\text{T}]\)leucine (Fig. 6B). The labeling intensities of the region A peptides in relation to the peptides outside region A confirm that region A contains serine-rich peptides. Furthermore, two additional serine-rich pVTG II peptides outside region A (indicated by stars in Fig. 6A) appear to contain little or no leucine (compare to Fig. 6B). Note that, in general, the serine-rich peptides of pVTG II do not have corresponding peptides of identical mobilities in the VTG II digest. In contrast, the leucine-rich peptides of pVTG II (Figs. 5 and 6B) outside region A have corresponding peptides of identical mobilities in the VTG II digest; these such peptides are indicated by arrows in Fig. 6. The lack of correspondence in the mobilities of the serine-rich peptides of pVTG II and VTG II may reflect differences in the protease cleavage sites as a result of the phosphates in VTG II. Alternatively, the phosphates of the VTG II peptides may alter the electrophoretic mobilities as occurs with intact vitellogenin. In either case, these data (Fig. 6) illustrate the nonuniform distribution of serine and leucine in the pVTG II and VTG II polypeptides. In addition, the serine-rich, but not the leucine-rich, peptides of VTG II appear to be modified so as to have altered electrophoretic mobilities in comparison to the serine-rich pVTG II peptides. This feature is consistent with the clustering of phosphates in the serine-rich region of VTG II as well as the influence of the phosphates upon the electrophoretic mobility of intact vitellogenin (vide infra).

Precursor Character of pVTG I and pVTG II—The precursor character of the pVTG was confirmed with both pulse-labeling and pulse-chase procedures. Fig. 7 illustrates the kinetics of \([\text{H}]\)serine incorporation into cellular vitellogenins when hepatocytes were analyzed after 15, 30, 60, and 120 min of labeling. With a 3-day film exposure, no labeling is detected at 15 min (Fig. 7, lane 1); only pVTG II is seen at 30 min (lane 2), pVTG I and pVTG II are seen at 60 min (lane 3), and pVTG I, pVTG II, and VTG II are seen at 120 min (lane 4). With an 11-week film exposure, the 15-min sample shows both pVTG I and pVTG II but not VTG II (lane 5). In contrast, the 120-min sample of similar intensity (lane 4) shows a distinct VTG II band as does the 30-min sample after an 11-week exposure (lane 6). In other experiments, we were unable to detect VTG I and VTG II among secreted proteins with labeling times of less than 30–35 min (data not shown). It appears, therefore, that \([\text{H}]\)serine is incorporated into the pVTG species prior to its appearance in either cellular or secreted VTG I and VTG II.

As noted above, only 2–6% of cellular vitellogenin is present as the VTG species with labeling times up to several hours. This result suggests that secreted VTG must arise directly from pVTG or via short lived cellular VTG intermediates. To determine the quantitative relationship between cellular pVTG and the secreted VTG species, hepatocytes were labeled with \([\text{H}]\)leucine for 40 min and chased with unlabeled leucine for 45 and 90 min. Cellular and secreted proteins were then analyzed by electrophoresis, and the radioactivity in the vitellogenin bands was determined by scintillation spectrometry after combustion of the gel slices to ensure quantitative recovery of radioactivity. No distinction was made between cellular pVTG and VTG species since the cellular VTG are such a small fraction of the total cellular vitellogenin. As shown in Table I, 90% of the cellular vitellogenin radioactivity was secreted during the 90-min chase. The extracellular vitellogenin recovered at each time point closely approximates that chased from the hepatocytes and can only be accounted for by the radioactivity initially present in the pVTG species. These data confirm that the secreted vitellogenins arise from cellular pVTG I and pVTG II.

Molecular Weight Estimations of the Vitellogenins—The large differences in the electrophoretic mobilities of the pVTG

\[
\begin{array}{ccc}
\text{VGT I} & \text{VGT II} & \text{pVTG I} & \text{pVTG II} \\
1 & 2 & 3 & 4 & 5 & 6 \\
\end{array}
\]

**Fig. 7.** Time course of \([\text{H}]\)serine incorporation into cellular vitellogenins. Hepatocytes were incubated with \([\text{H}]\)serine for 15 min (lanes 1 and 5), 30 min (lanes 2 and 6), 60 min (lane 3), and 120 min (lane 4). Cells were washed, solubilized in SDS sample buffer, and run on an SDS-5% polyacrylamide slab gel. Plasma VTG I and VTG II were run in an adjacent gel lane. Radioactive proteins were visualized by fluorography with exposures of 3 days (lanes 1–4) and 11 weeks (lanes 5 and 6).

**Table I**

| Chase time | Cellular | Secreted |
|------------|----------|----------|
| Min        | cpm/10^6 cells |          |
| 0          | 136,100 ± 9,390 | 850 ± 30 |
| 45         | 120,700 ± 2,000 | 16,800 ± 1,100 |
| 90         | 94,100 ± 1,440 | 47,400 ± 1,400 |
| Δ 0–45     | –15,400 | +15,950 |
| Δ 0–90     | –42,000 | +46,550 |

Biosynthesis of the Vitellogenins

---

**Table I**

| Chase time | Cellular | Secreted |
|------------|----------|----------|
| Min        | cpm/10^6 cells |          |
| 0          | 136,100 ± 9,390 | 850 ± 30 |
| 45         | 120,700 ± 2,000 | 16,800 ± 1,100 |
| 90         | 94,100 ± 1,440 | 47,400 ± 1,400 |
| Δ 0–45     | –15,400 | +15,950 |
| Δ 0–90     | –42,000 | +46,550 |
judged by the incorporation of radiophosphate during in vivo dephosphorylation for 0 min, 10 min, 20 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, and 5 h were run in lanes 1-10, respectively, of an SDS-5% polyacrylamide slab gel (left). Protein was visualized by staining with Coomassie blue. The gel was then prepared for fluorography to visualize the cellular 

3-10, TgG and pVTG species suggest that SDS-polyacrylamide gel electrophoresis is an inappropriate method to estimate the molecular weights of the vitellogenin polypeptides. We have, therefore, explored the influence of the phosphates upon the mobility of plasma vitellogenin and estimated the molecular weights of the various vitellogenin species by an independent method. Fig. 8 shows the results of an experiment in which a plasma vitellogenin sample enriched in VTG II was progressively dephosphorylated. The electrophoretic profile of the untreated sample (Fig. 8, left, lane 1) is shown in comparison to the mobilities of the VTG and pVTG species. As VTG II was dephosphorylated (lanes 2-10), the band broadened and its mobility increased until the leading edge had the mobility of pVTG II. This mobility shift closely parallels dephosphorylation as judged by the release of vitellogenin phosphorus (Fig. 8, right). The dephosphorylation and mobility shift were maximal by 2 h and remained unchanged for a further 3 h (lanes 7-10). In other experiments employing successive treatments with alkaline phosphatase, the mobility of the leading edge of the VTG II band did not increase further, but the band became much sharper. Similar results have been obtained with plasma VTG I in which case the dephosphorylated protein had the same mobility as pVTG I (data not shown). In addition, note that two very faint bands (Fig. 8, left) which run slightly ahead of the VTG II band also shift in mobility during treatment with alkaline phosphatase. We have determined that these proteins are also heavily phosphorylated as judged by the incorporation of radiophosphate during in vitro labeling experiments (data not shown). These results indicate that the phosphates markedly influence the mobility of vitellogenin in SDS-polyacrylamide gels.

Independent estimates of vitellogenin molecular weight were obtained by gel chromatography of reduced and alkylated proteins using 7 M guanidine-HCl as solvent. Fig. 9 shows a calibration curve which employed β-galactosidase, myosin, and human apolipoprotein B (22) as high molecular weight standards. Plasma VTG II and dephosphorylated VTG II showed elution volumes corresponding to the \( M_r = 170,000 \) to 190,000 range. When the mixture of VTG I and VTG II was analyzed, both proteins eluted together in the same molecular weight range. Hepatocyte proteins labeled with \(^{3}H\)serine were analyzed in a similar fashion. Subsequent to chromatography, column fractions were analyzed by SDS-5% polyacryl-
inert cellular vitellogenins seen whether hepatocytes or tissue slices were boiled directly in SDS electrophoresis sample buffer (as in Fig. 1, lane 1) or homogenized to prepare a cell extract for immunochemical procedures (as in Fig. 2, lanes 1 and 3). This was also the case when cells or tissue slices were homogenized directly in trichloroacetic acid. Second, the predominance of the cellular pVTG species was unaltered by a variety of protease inhibitors (see “Experimental Procedures”) whether the inhibitors were included in homogenization buffer for cell extract preparation or in electrophoresis sample buffer used for direct solubilization of hepatocytes. Third, mixing experiments were carried out in which medium containing [H]serine-labeled VTG I and VTG II was included in buffer used to homogenize unlabeled hepatocytes. No breakdown of the [H]-vitellogenin was seen upon subsequent electrophoresis (data not shown). These data eliminate the remote possibility that the pVTG species arose through proteolytic cleavage of VTG I and VTG II.

**DISCUSSION**

The experiments reported here identify and characterize discrete intracellular vitellogenins, pVTG I and pVTG II, and establish that they give rise to secreted vitellogenins. The pVTG are identified as vitellogenins by specific reactivity with antibodies to the plasma vitellogenins (Figs. 2–4). In addition, pVTG I and pVTG II are made by hepatocytes from laying hens and estrogen-stimulated roosters but not by hepatocytes from unstimulated roosters. Furthermore, pVTG I and pVTG II are clearly intracellular species which do not appear in proteins secreted by hepatocytes (Fig. 1, lane 2) or in plasma of estrogen-stimulated roosters (7). The precursor character of the pVTG is established by three findings. First, pVTG I and pVTG II contain little or no phosphorus (Fig. 2), indicating that they are intermediates in the biosynthesis of the mature phosphorylated vitellogenins. Second, precursor amino acid is incorporated into pVTG I and pVTG II prior to its appearance in cellular and secreted vitellogenins when hepatocytes are continuously incubated with [H]serine (Fig. 7). Third, quantitative analysis of vitellogenin secretion during a pulse-chase experiment indicates that only the cellular pVTG can account for the secreted vitellogenins (Table I).

The identification of pVTG II as the specific precursor to secreted VTG II is strongly supported by both immunological and peptide map comparisons. Anti-VTG II selectively precipitates pVTG II as well as cellular and secreted VTG II but is far less effective in precipitating pVTG I. This is in agreement with previous work which showed poor reactivity between purified plasma VTG I and anti-VTG II (7). Partial proteolysis mapping of [H]leucine and [H]serine-labeled vitellogenins shows extensive similarity between pVTG I and anti-VTG II (Figs. 5 and 6) and illustrates the nonuniform distribution of serine and leucine in the pVTG II and VTG II polypeptides. Although the data are less extensive, similar structural relatedness is evident in the proteolysis products of pVTG I and VTG I (Fig. 5). The dissimilarity of the pVTG I and pVTG II digestion products as well as the poor reactivity of pVTG I with anti-VTG II suggests that pVTG I is specifically related to secreted VTG I but not VTG II.

The large differences in the mobilities of the pVTG and VTG species as well as the increased mobility of dephosphorylated plasma vitellogenin indicate that SDS-polyacrylamide gel electrophoresis does not accurately estimate the molecular weights of these heavily phosphorylated proteins. Estimates of vitellogenin molecular weight by this method in numerous laboratories (1, 7, 8, 34, 35) yield values of 235,000 to 260,000. In contrast, the mobilities of pVTG I and VTG II correspond to \( M_r \approx 200,000 \) and 190,000. A more accurate estimate by gel chromatography in 7 M guanidine-HCl confirms that vitellogenin molecular weight is much less than estimated by SDS-polyacrylamide gel electrophoresis. The various vitellogenin species are not resolved by this chromatographic method, but all species showed \( M_r \approx 170,000 \) and 190,000 with a mean of approximately 180,000 (Fig. 9). The molecular weights of the pVTG species as estimated by electrophoresis exceed this value by only about 10%, a difference which might be explained by the carbohydrate content of these proteins. In studies to be reported elsewhere, we have determined that the plasma vitellogenins contain approximately 1% carbohydrate. It is unlikely that this low carbohydrate content would significantly influence estimations by chromatography in guanidine-HCl (36), although some retardation in mobility might be expected in SDS-polyacrylamide gels (37). Indeed, we have noted that the pVTG species made in the presence of the glycosylation inhibitor tunicamycin (30, 31) have slightly greater gel mobilities yielding \( M_r \approx 190,000 \) and 180,000 for nonglycosylated pVTG I and pVTG II, respectively. These findings further confirm that the vitellogenin polypeptides have \( M_r \approx 180,000 \).

Cell-free translations of intact, highly purified vitellogenin mRNA have also shown that the cell-free product has a greater mobility on SDS-polyacrylamide gels than plasma vitellogenin. In the cases of avian (38, 39) and amphibian (40) vitellogenins, the greater mobilities of the cell-free products were attributed to the lack of post-translational modifications such as phosphorylation and glycosylation. Our results on the markedly different electrophoretic mobilities of the nonphosphorylated and phosphorylated vitellogenins support these interpretations. The report by Gordon et al. (41) that nonphosphorylated vitellogenin synthesized in a cell-free system has the same electrophoretic mobility as plasma vitellogenin is likely due to the use of an acrylamide concentration which is too high to permit resolution of proteins in this molecular weight range. We find, for example, that the pVTG and VTG bands are not resolved in SDS-7.5% polyacrylamide gels, particularly when the vitellogenin is run into the gel for only a short distance. Even with an SDS-5% polyacrylamide gel, the pVTG and VTG species are well resolved only with extended electrophoresis times such that proteins of \( M_r = 100,000 \) have migrated approximately 8–10 cm (Fig. 1).

A polypeptide weight of 180,000 has significant implications for the analysis of vitellogenin processing in the oocyte. After secretion from the liver and uptake into the developing oocyte, the avian vitellogenins give rise to yolk proteins designated \( \alpha \)-lipovitellin, \( \beta \)-lipovitellin, and phosvitins. Both lipovitellins contain two or more polypeptide chains which arise from vitellogenin regions containing little phosphorus while the heavily phosphorylated vitellogenin regions give rise to at least two phosvitins (1, 8, 32, 42). The organization of the various yolk polypeptides within the vitellogenin polypeptides is not well understood. We have previously presented evidence that VTG II is the precursor to a single yolk phosphoprotein as well as the 125,000-dalton polypeptide of \( \beta \)-lipovitellin (7). The \( \beta \)-lipovitellin monomeric mass of 200,000 includes 160,000 daltons accounted for by protein (43, 44) which can be resolved into equimolar quantities of the 125,000- and 30,000-dalton polypeptides (1, 8, 42). A VTG II polypeptide of 180,000, therefore, could include both \( \beta \)-lipovitellin polypeptides with approximately 25,000 daltons remaining to accommodate the phosvitin region of the molecule. After correction for phosphorus (32, 45) and carbohydrate (46), the polypeptides of either the \( M_r = 34,000 \) or 28,000 phosvitin (32), but not both phosvitins, could be accommodated. Comparison of phosphorus contents also confirms that only one phosvitin can be present in VTG II. With a phosphorus content of 2% of
polypeptide weight (7), VTG II contains approximately 116 mol of phosphorus. Within the error of these measurements, this is sufficient to account for the 117-127 mol of phosphorus present in β-lipovitellin (47) plus either phosvitin (32) but far too little to account for the 217 mol of phosphorus in β-lipovitellin plus both phosvitins. Similar calculations indicate that VTG I also contains only one phosvitin. These data, therefore, support the view (7) that each avian vitellogenin polypeptide contains a phosphoserine-rich region which gives rise to one or the other of the two yolk phosvitins.

The experiments described here are of particular interest with regard to the biosynthesis and post-translational modifications of the vitellogenins. These experiments resolve, identify, and characterize individual intracellular vitellogenins and biosynthetic intermediates which appear to represent discrete stages of post-translational processing. Such analyses are necessary in order to understand the biosynthesis of these complex proteins, but have not been reported previously. The pVTG are glycosylated vitellogenins containing little or no phosphorus, indicating that some glycosylation reactions occur prior to phosphorylation. While it is not clear whether glycosylation is initiated during or after polypeptide synthesis, it is clear that phosphorylation occurs after completion of the vitellogenin polypeptide and after the addition of some carbohydrate. The question of whether carbohydrate plays a role in targeting which serines are phosphorylated can be raised since yolk phosvitin contains a glycopeptide sequence that includes a block of eight phosphoserines (46). With the caveat that glycosylation may be initiated during polypeptide synthesis, the stages of vitellogenin biosynthesis may be ordered as: polypeptide synthesis → glycosylation → phosphorylation → secretion.

The phosphorylation of each vitellogenin requires the addition of over 100 phosphates/polypeptide chain. The gradual mobility shift accompanying vitellogenin dephosphorylation (Fig. 8) suggests that partially phosphorylated vitellogenins would have mobilities intermediate to the mobilities of the pVTG and VTG species. Interestingly, significant amounts of vitellogenins with intermediate mobilities were not seen after labeling with [3H]serine (Figs. 1A and 2). Even after labeling with [32P], for times as brief as 1 min, significant amounts of such intermediates are not seen.5 The absence of such intermediates suggests that once the phosphorylation of the vitellogenin polypeptide begins, it is rapidly completed. One possibility consistent with this observation is that once the phosphorylation of the vitellogenin to enter or pass through a cell compartment that contains a high kinase concentration and represents only a small fraction of the intracellular transport pathway. Additionally, if vitellogenin is vectorially transported (48), the subcellular site of phosphorylation must be very near the terminal stages of the pathway since the majority of the intracellular vitellogenin is present as nonphosphorylated pVTG I and pVTG II. It appears that when phosphorylation is completed, the vitellogenins are rapidly secreted from the hepatocyte. Consistent with this interpretation is the observation that [32P]vitellogenin appears in plasma with a lag of only several minutes after [32P]administration to roosters (49). Schirm et al. (50) have also shown that [32P]vitellogenin accumulates in plasma after inhibition of protein synthesis. These data also suggest that vitellogenin phosphorylation occurs immediately prior to secretion. Although the subcellular site and mechanism of vitellogenin phosphorylation are not known, the ability to monitor the intracellular pVTG and VTG species should greatly facilitate such studies. Further-

more, the pVTG species are an appropriate substrate for the analysis of vitellogenin phosphorylation in cell-free systems.

Addendum—Wiley and Wallace (51) have recently presented evidence that in Xenopus laevis the multiple yolk lipovitellin polypeptides and the various phosphoproteins are derived from multiple forms of plasma vitellogenin. There is thus agreement that the heterogeneity of yolk polypeptides in both species is due to heterogeneity in the vitellogenin precursors.

REFERENCES

1. Bergink, W. E., Wallace, R. A., Van de Berg, J. A., Bos, E. S., Gruber, M., and AB, G. (1974) Am. Zool. 14, 1177-1183

2. Tata, J. R., and Smith, D. F. (1979) Rec. Prog. Horm. Res. 35, 47-90

3. Ryffel, G. U. (1978) Mol. Cell. Endocrinol. 12, 237-248

4. Wahli, W., Dawid, I. B., Wyler, T., Jaggi, R. B., Weber, R., and Ryffel, G. U. (1979) Cell 16, 535-549

5. Wahli, W., Dawid, I. B., Ryffel, G. U., and Weber, R. (1981) Science 211, 298-304

6. Wiley, H. S., and Wallace, R. A. (1978) Biochem. Biophys. Res. Commun. 85, 153-159

7. Wang, S.-Y., and Williams, D. L. (1980) Biochemistry 19, 1557-1563

8. Christmann, J. L., Grayson, M. J., and Huang, R. C. C. (1977) Biochemistry 16, 3250-3256

9. Williams, D. L., Tseng, M. T., and Rottmann, W. (1977) Life Sci. 23, 195-206

10. Seglen, P. O. (1976) Methods Cell Biol. 13, 29-83

11. Williams, D. L. (1979) Biochemistry 18, 1066-1069

12. Umbleit, W. W., Burris, R. H., and Stauffer, J. F. (1945) in Manometric Techniques and Tissue Metabolism, p. 119, Burgess Publishing Co., Minneapolis MN

13. Laemmli, U. K. (1970) Nature (Lond.) 227, 680-685

14. Bonner, W. M., and Laskey, R. A. (1974) Eur. J. Biochem. 46, 518

15. Cleveland, D. W., Fischer, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) J. Biol. Chem. 252, 1102-1106

16. Chen, P. S., Toribara, T. Y., and Warner, H. (1956) Anal. Chem. 28, 1756-1758

17. Means, G. E., and Feneely, R. E. (1971) Chemical Modification of Proteins, p. 219, Holden-Day, San Francisco, CA

18. Mann, K. G., and Fish, W. W. (1972) Methods Enzymol. 26, 28-42

19. Ackers, G. K. (1967) J. Biol. Chem. 242, 3237-3238

20. Fish, W. W., Mann, K. G., and Tanford, C. (1989) J. Biol. Chem. 264, 4989-4994

21. Havel, R. J., Eder, H. A., and Bragdon, J. H. (1955) J. Clin. Invest. 34, 1345-1353

22. Steele, J. C. H., Jr., and Reynolds, J. A. (1979) J. Biol. Chem. 254, 1639-1643

23. Jovin, T. Y., Ohno, T., Panyim, S., and Schuerr, A. R. (1978) Eur. J. Biochem. 84, 355-361

24. Burns, A. T. H., Deeley, R. G., Gordson, J. I., Udell, D. S., Mullinix, K. P., and Goldberger, R. F. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 1815-1819

25. Williams, D. L., Wang, S.-Y., and Klett, H. 1978) Proc. Natl. Acad. Sci. U. S. A. 75, 5974-5978

26. Williams, D. L., Wang, S.-Y., and Capony, F. (1979) J. Steroid Biochem. 11, 231-236

27. Nadin-Davis, S. A., Lazier, C. B., Capony, F., and Williams, D. L. (1980) Biochem. J. 192, 733-740

28. Capony, F., and Williams, D. L. (1980) Biochemistry 19, 2219-2226

29. Blue, M.-L., Procter, A. A., and Williams, D. L. (1980) J. Biol. Chem. 255, 10048-10051

30. Tkacz, J. S., and Lampen, J. O. (1975) Biochem. Biophys. Res. Commun. 65, 248-257

31. Struck, D. K., and Lennard, W. J. (1977) J. Biol. Chem. 252, 1007-1013

32. Clark, R. C. (1970) Biochem. J. 118, 537-542

33. Cook, W. H., Burley, R. W., Martin, W. G., and Hopkins, J. W. (1980) Biochem. Biophys. Acta 60, 98-103

34. Deeley, R. G., Mullinix, K. P., Wetekam, W., Kronenberg, H. M., Meyers, M., Eldridge, J. D., and Goldberger, R. F. (1975) J.

5 S. Y. Wang, and D. L. Williams, unpublished observation.
3846 Biosynthesis of the Vitellogenins

Biol. Chem. 250, 9060–9066

35. Lazier, C. B. (1978) Biochem. J. 174, 143–152
36. Leach, B. S., Collawn, J. F., Jr., and Fish, W. W. (1980) Biochemistry 19, 5741–5747
37. Leach, B. S., Collawn, J. F., Jr., and Fish, W. W. (1980) Biochemistry 19, 5734–5741
38. AB, G., Roskam, W. G., Dijkstra, J., Mulder, J., Willems, M., Van Der Ende, A., and Gruber, M. (1976) Biochim. Biophys. Acta 454, 67–78
39. Wieringa, B., Mulder, J., Van Der Ende, A., Bruggeman, A., AB, G., and Gruber, M. (1978) Eur. J. Biochem. 89, 67–79
40. Felber, B. K., Maurhofer, S., Jaggi, R. B., Wyler, T., Wahli, W., Ryffel, G. U., and Weber, R. (1980) Eur. J. Biochem. 105, 17–24
41. Gordon, J. I., Deeley, R. G., Burns, A. T. H., Paterson, B. M., Christmann, J. L., and Goldberger, R. F. (1977) J. Biol. Chem. 252, 8320–8327
42. Bos, E. S. (1975) Ph.D. thesis, The University of Groningen, Groningen, The Netherlands
43. Bernardi, G., and Cook, W. H. (1960) Biochim. Biophys. Acta 44, 96–105
44. Bernardi, G., and Cook, W. H. (1960) Biochim. Biophys. Acta 44, 105–109
45. Allerton, S. E., and Perlman, G. E. (1965) J. Biol. Chem. 240, 3892–3898
46. Shainkin, R., and Perlmann, G. E. (1971) J. Biol. Chem. 246, 2278–2284
47. Wallace, R. A. (1965) Anal. Biochem. 11, 297–311
48. Palade, G. E. (1975) Science 189, 347–358
49. Bergink, E. W., Kloosterboer, H. J., Gruber, M., and AB, G. (1973) Biochim. Biophys. Acta 294, 497–506
50. Schirm, J., Gruber, M., and AB, G. (1973) FEBS Lett. 30, 167–169
51. Wiley, H. S., and Wallace, R. A. (1981) J. Biol. Chem. 256, 8628–8634
Biosynthesis of the vitellogenins. Identification and characterization of nonphosphorylated precursors to avian vitellogenin I and vitellogenin II.
S Y Wang and D L Williams

J. Biol. Chem. 1982, 257:3837-3846.

Access the most updated version of this article at http://www.jbc.org/content/257/7/3837

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/257/7/3837.full.html#ref-list-1