Regulation of Protein Synthesis in Chick Oviduct

I. INDEPENDENT REGULATION OF OVALBUMIN, CONALBUMIN, OVOMUCOID, AND LYSOZYME INDUCTION*

(Received for publication, April 19, 1972)

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SUMMARY

Egg white protein synthesis is induced and maintained in oviduct magnum of immature female chicks by administering gonadal steroid hormones. The relative rate of synthesis of four egg white proteins (ovalbumin, conalbumin, ovomucoid, and lysozyme) was measured by culturing magnum explants with radioactive amino acids followed by specific immunoprecipitation of each of the egg white proteins. Induction of these proteins was studied as a function of time, hormonal dose, and hormonal combination. A combination of estrogen, progesterone, and testosterone was found which would induce the relative rate of synthesis of each of the four egg white proteins to a level that closely approximates that observed in laying hens; this combination also promotes maximal magnum growth.

The synthesis of these proteins is not strictly coordinated, since the rate of synthesis of one or more of these proteins can change relative to the others, although they are all synthesized in the same cell. Noncoordinate protein synthesis is exemplified by the changing ratio of conalbumin to ovalbumin synthesis during both primary and secondary stimulation with estrogen. The ratio of synthesis of these two proteins also changes as a function of the dosage of hormone administered. Furthermore, different combinations of hormones can produce noncoordinate synthesis; for example, the synthesis of ovomucoid and conalbumin is increased relative to ovalbumin when either progesterone or testosterone are administered along with estrogen. The most likely explanation for the change in the ratio of ovalbumin to conalbumin synthesis observed during hormonal stimulation is the 2-fold difference in the rates of degradation of their mRNAs. In contrast, changes in mRNA synthesis or activation best account for the preferential induction of conalbumin synthesis by low doses of estrogen, and the increased rates of conalbumin and ovomucoid synthesis which are produced by supplementing estrogen with either progesterone or testosterone.

The results suggest that a single regulatory site for the control of egg white synthesis is unlikely since several of these proteins appear to be under independent control.

An unanswered question about the regulation of protein synthesis in eukaryotic organisms is whether there is any equivalent to the bacterial operon, such that the induction of several proteins is strictly coordinated by a common regulatory factor. The induction of egg white proteins was used to test this possibility because several of these proteins are synthesized in the same cell and because these proteins are packaged so that eggs with a fairly constant composition are produced. Thus, the question is whether the synthesis of the mRNAs for each of these proteins is coordinate, i.e. in constant proportion to one another. Since techniques were not available to measure coordinate synthesis of the mRNAs directly, the translatable mRNA concentrations were estimated by measuring the relative rates of synthesis of the proteins for which they encode. Relating protein synthesis to mRNA synthesis must be considered tentative because of the many potential regulatory steps between these two events. For instance, the relative rate of protein synthesis is related to mRNA concentration only if all mRNAs are translated at the same rate. Furthermore, the concentration of mRNAs is not only a function of synthesis but also of activation and degradation. After taking the above considerations into account, the results suggest that several of the egg white proteins are regulated independently.

* This work was supported in part by Research Grant GM14931 from National Institute of General Medical Studies, and Research Grant P-427C from the American Cancer Society.
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rather than strictly coordinately. However, there are many aspects of the synthesis of egg white proteins which indicate that their induction is interrelated and under similar control.

**METHODS**

**Hormone Injection Regimen—**Hormones were administered to 4- to 10-day-old, female, White Leghorn chicks according to a standardized regimens consisting of an optimal dose of estrogen (1 mg) administered daily for 10 days (a period called primary stimulation), then 15 to 30 days without hormone administration (called withdrawal), followed by several days of secondary stimulation with various steroid hormones, at a dose of 2 mg per day unless otherwise noted. A dose of 2 mg per day was chosen because that dose of estrogen, progesterone, or testosterone produces maximal effects when administered to 200- to 400-g chicks (5, 6, 9). The effect of this regimen on various magnum parameters, viz. growth, protein concentration and rate of synthesis, ribosome concentration and aggregation into polysomes, has been published (10).

17β-Estradiol benzoate was a gift from Schering; 2-α-methyl dihydrotestosterone propionate was a gift from Syntax; progesterone was purchased from Schering as “Prolutan.” All hormones were dissolved in sesame oil at a concentration of 10 mg per ml (10 min in a boiling water bath was required to dissolve the estrogen) and injected into the lower leg muscles.

**Purification of Antigen—**The protein peaks from the columns described below were localized by measuring the absorption at 280 nm and samples from the center and periphery of the peaks were subjected to electrophoresis as in Fig. 1. Only those fractions which were uncontaminated were pooled and concentrated. The extinction coefficients and approximate molecular weights are given in Table I.

Ovalbumin (five times crystallized, Nutritional Biochemicals) was purified on DEAE-cellulose as previously described (13). Only the A, form of ovalbumin which carries 2 phosphate residues was used as antigen (Fig. 1).

Conalbumin was purified by modifying the method of Warner (13). Egg whites were blended for 40 s in a Waring Blender, filtered through glass wool, and centrifuged 10 min at 13,000 × g. Ethanol was added to the supernatant to 20% and the temperature lowered to 0°. The precipitate which formed was removed by centrifugation and resuspended in 20 mM glycine, dialyzed against 20 mM glycine overnight, applied to a DEAE-cellulose column, and eluted with the buffer system proposed by Mandeles (14). The peak fractions containing conalbumin were concentrated by ammonium sulfate precipitation (70%), dialyzed against distilled water, and frozen. Fig. 1 shows the electrophoresis of conalbumin in two systems.

Ovomucoid was purified from egg white trypsin inhibitor (Sigma). The crude protein was dissolved in 10 mM sodium phosphate, pH 7.4, and applied to a DEAE-cellulose column equilibrated with the same buffer. After washing with 160 ml of phosphate buffer a gradient of NaCl from 0 to 0.8 M was started. The peak ovomucoid fractions were pooled, the pH adjusted to 3.5 with HSO₄, and then an equal volume of 10% trichloroacetic acid (pH 3) was added. The precipitate which formed was removed by centrifugation and discarded. The supernatant was dialyzed against distilled water and lyophilized. Since ovomucoid does not precipitate with trichloroacetic acid it diffused out of the acrylamide gels during the destaining procedures following electrophoresis and therefore is not shown in Fig. 1. However, the protein could be detected as a diffuse band in the gels during the early stages of destaining; the mobility of this band was similar to ovalbumin in SDS gels and migrated approximately halfway between ovalbumin and conalbumin in the pH 8.9 gels shown in Fig. 1. In neither case was the presence of any other protein detected.

Lysozyme (three times crystallized, Nutritional Biochemicals) was further purified with methods adapted from Warner (13). The protein was dissolved in 10 mM sodium phosphate, pH 7.4, and applied to a CM-cellulose column equilibrated with the same buffer and eluted with a gradient of NaCl up to 0.5 M. The peak lysozyme fractions were adjusted to pH 10 and NaCl added to 1 M. The precipitate which formed was collected by centrifugation, then dissolved in, and dialyzed against, distilled water, and finally lyophilized. Lysozyme migrates toward the cathode in pH 8.9 and hence the electrophoresis of this protein is only shown in the SDS gels (Fig. 1).

Bovine serum albumin (crystalline, Pentex) was used without further purification.

**Antibody Preparation—**Rabbits were injected subcutaneously with approximately 5 mg of purified antigen dissolved in 1 ml of distilled water and sonicated with an equal volume of Freund's complete adjuvant. The rabbits were bled several weeks later and a crude γ-globulin fraction prepared by ammonium sulfate precipitation as before (12). Rabbits were given booster immunizations when necessary. As a precaution, purified ovalbumin, or conalbumin, or both, were added to all nonimmune sera prior to ammonium sulfate fractionation and any precipitate which formed was removed by centrifugation. The approximate titre of the antibody preparations used, expressed as micrograms of γ-globulin necessary to precipitate 1 μg of antigen at the equivalence point, is given in Table I.

**Labeling Oviduct Proteins and Preparation of Homogenates—**Oviducts were removed from decapitated chicks with sterile procedures, the magnum portion isolated and cut into four to ten pieces. These were incubated in Hanks' balanced salt solution containing penicillin and streptomycin, buffered with NaHCO₃, and continuously gassed with 95% O₂-5% CO₂ as previously described (12). The explants were labeled with either ²H-amino acid mixtures (125 μCi per ml, Schwarz) or ³⁵S-amino acid mixtures (125 μCi per ml, Schwarz) for 1 to 3 hours. After labeling,

![Fig. 1. Acrylamide gel electrophoresis of egg white proteins.](image-url)
the explants were blotted, weighed, and a 10% homogenate prepared in 25 mM Tris, 25 mM NaCl, 5 mM MgCl₂, 1% Triton X-100, 1% sodium deoxycholate, pH 7.6, with a motor-driven glass wool if much lipid was present and used in relative rates of ovalbumin synthesis about 1.6 times higher with the above buffer without detergents and centrifuged 90 min at 40,000 rpm (Spinco 40 rotor). The supernatants were needed to precipitate 1 µg of antigen (Ag) at equivalence point assuming an ε_{280nm} of 14 for the γ-globulin.

### Immunoprecipitation

Immunoprecipitation of radioactive oviduct proteins was performed essentially as before (12). At least two different dilutions of supernatant were always precipitated to ensure antibody excess, and 5 µg of unlabelled purified antigens were added to all samples which did not contain at least that amount of endogenous antigen (immunoprecipitation of [3H]-ovalbumin was incomplete when less than 4 µg of ovalbumin was present). The precipitation of 5 µg of bovine serum albumin added to the radioactive oviduct homogenate by anti-bovine serum albumin was used as a control for nonspecific coprecipitation. This value (usually 0.2 ± 0.1% of total acid-precipitable radioactivity) was determined for each homogenate and subtracted from the experimental values. Total acid-precipitable radioactivity was determined as before (8). The counting efficiency for both acid precipitates and immunoprecipitates was between 50 to 55% for [3H] and 80 to 85% for [14C].

### RESULTS

#### Validity of Methods

**Labeling Oviduct Proteins**—Synthesis of magnum protein in culture has been shown to be qualitatively and quantitatively similar to that in vivo (12). Over 95% of the incorporated amino acids are recovered in the tissue when labeling times are less than 5 hours; and the homogenization procedures used solubilize more than 90% of the newly synthesized protein. Thus, measuring relative rates synthesis of specific protein in culture probably reflects the in vivo condition.

The radioactive profile of magnum proteins separated by SDS-acrylamide gel electrophoresis from chickens given 8 days of primary stimulation and from chicks after several weeks of withdrawal are shown in Fig. 2 (left panel). The profile of magnum proteins from withdrawn chicks is similar to that from unstimulated chicks (8). Estrogen treatment changes completely the profile of proteins synthesized; the two major peaks correspond to conalbumin and ovalbumin.

**Antibody Specificity**—The specificity of each antibody preparation used was ascertained by SDS-acrylamide gel electrophoretic analysis of the immunoprecipitates formed when an excess of antibody was added to a magnum homogenate of tissue which had been labeled in culture with radioactive amino acids (12). Fig. 2 shows the specificity of the anti-ovalbumin, anti-conalbumin, anti-ovomucoid, and anti-lysozyme preparations. In each case the precipitate was formed in [3H]-labeled homogenate and [14C]ovalbumin was added as an internal marker. Each panel shows a single tritium radioactive peak which accounts for at least 90% of the total immunoprecipitable radioactivity.

All the proteins migrated according to the log of their molecular weight (Table I) except ovomucoid. The anomalous migration of ovomucoid may be due to the fact that this protein contains approximately 15% carbohydrate (15). Since the mobility of ovomucoid is the same as ovalbumin on SDS-acrylamide gels, the specificity of the anti-ovomucoid preparation was also tested.
Immunological determination of relative rate of specific egg white protein synthesis. Varying amounts of 3H-labeled homogenate from 8-day primary stimulated magnum (Fig. 2) were added to excess specific antibody and the radioactivity in washed precipitates was determined as before (12). Anti-bovine serum albumin (BSA) was used as a control (see "Methods"). The relative rate of synthesis in parentheses was determined by dividing the immunoprecipitated radioactivity by that precipitated with trichloroacetic acid (50,000 cpm/100 µl). anti-OV, anti-ovalbumin; anti-CON, anti-conalbumin; anti-MU, anti-ovomucoid; anti-LYS, anti-lysozyme.

for its ability to precipitate varying amounts of purified [3H]-ovalbumin. Ovalbumin was not precipitated.

Measuring Relative Rate of Specific Protein Synthesis—The rates of egg white protein synthesis are expressed as relative rates of protein synthesis, i.e. the percentage of the total acid-precipitable radioactivity which is specifically immunoprecipitable. This form of data presentation was chosen because: (a) it eases the comparison of one tissue with another by avoiding the problems inherent in measuring the actual rates of protein synthesis which entails knowing the specific activity of the amino acid pools; and (b) the relative rate of protein synthesis may be directly related to the relative mRNA concentration if the rates of polypeptide initiation and elongation on all mRNAs are the same (see "Discussion").

Fig. 3 illustrates the method used to measure the relative rate of synthesis of four of the egg white proteins. The amounts of supernatant used for each curve depend on the endogenous antigen concentration and the antibody titres. After 8 days of primary stimulation with estrogen the relative rates of synthesis of each of the proteins, except ovomucoid, approximate the relative amount of each of the proteins in egg white (Table I). Together these four proteins account for about 68% of total protein synthesis after this hormonal treatment.

Induction of Egg White Protein Synthesis with Estrogen

Time Course—The relative rates of ovalbumin and conalbumin synthesis at various times during a standardized regimen of hormonal stimulation and withdrawal are shown in Fig. 4. Ovalbumin synthesis is undetectable in unstimulated immature chicks and is first detectable approximately 24 hours after primary stimulation (8). Fig. 4 (top) shows that with continued estrogen administration, ovalbumin synthesis increases until it accounts for 30 to 60% of the total protein being synthesized in explanted magnums after 10 days of primary stimulation. During withdrawal the relative rate of ovalbumin synthesis declines until it is again undetectable after 10 to 15 days. Light and electron micrographs, and measurements of DNA content, indicate that during withdrawal most of the tubular gland cells are retained although they cease to synthesize the characteristic secretory proteins (6, 10). After commencing secondary stimulation, ovalbumin synthesis rises to the same maximal value as during primary stimulation but in about one-half the time. In addition, there is only a 5-hour lag before ovalbumin is first detectable after secondary stimulation.

Fig. 4 (bottom) shows that the rate of conalbumin synthesis reaches a value of 10 to 12% of the total protein being synthesized but this value is reached in about one-half the time it takes ovalbumin to reach its maximal values. Also, the rate of decay of conalbumin synthesis during withdrawal is slower than that of ovalbumin synthesis. Thus, the ratio of ovalbumin to conalbumin synthesis changes from about 1 during early primary and secondary stimulation and late withdrawal to approximately 6 during late primary and secondary stimulation. The maximal ovalbumin to conalbumin synthesis ratios are similar to that in magnum tissue from laying hens (16) and resemble the ratio of these proteins in egg white (Table I). Possible mechanisms underlying the noncoordinate synthesis of these two proteins are examined below.

Estrogen Dose Response—The effect of varying doses of estrogen during secondary stimulation on the relative rate of specific protein synthesis, as well as magnum growth, is shown in Fig. 5. After either 1 day (Fig. 5A) or 2 days (Fig. 5B) of half-maximal stimulation of specific protein synthesis and growth occurs with doses of 0.1 to 0.2 mg of estrogen per day to 400-g chicks. Maxi-
both primary and secondary stimulation there are times and half-lives of their mRNAs (discussed below). The reason for this discrepancy is that the estrogen concentration in the serum may decline slowly during withdrawal and may be insufficient to maintain secretory protein synthesis for some time. This explanation is possible because estrogen is administered as a supersaturated solution in oil, thus creating a depot for slow release.

This possibility was tested by inducing egg white protein synthesis with a lower dose of estrogen (0.1 µg per day) and then measuring the relative rate of ovalbumin and conalbumin synthesis during withdrawal (Fig. 7). Comparison of the results with those in Fig. 4 shows that conalbumin was induced to the same extent with either dose of estrogen, whereas ovalbumin was in-
duced to only 25% of total protein synthesis with the lower dose of estrogen compared to almost 60% with the higher dose, a result consistent with those discussed above. The relative rate of both ovalbumin and conalbumin synthesis declines faster after induction with the lower dose of estrogen; the \( t_{1/2} \) of ovalbumin relative rate of synthesis is reduced from 4 days to less than 1 day, while the \( t_{1/2} \) of conalbumin synthesis falls from 8 days to about 1 day.

**Induction of Egg White Proteins with Estrogen, Progesterone, and Testosterone Alone, and in Various Combinations**

**Estrogen Alone**—This hormone induces the synthesis of all the egg white proteins measured during primary and secondary stimulation (Figs. 3, 4, 9). Induction with estrogen will be used as a standard for comparison in the sections below.

**Testosterone Alone**—Testosterone does not induce the synthesis of any of the egg white proteins tested when given alone, nor does it affect magnum growth (Table II). The values given are from chicks given 4 days of secondary stimulation; they are not significantly different from those obtained in control chicks (29 days withdrawn).

**Progesterone Alone**—Although incapable of inducing ovalbumin synthesis (and presumably the synthesis of conalbumin, ovomucoid, and lysozyme as well) during primary stimulation (8), progesterone induces the synthesis of all four proteins when administered as a secondary stimulation (Table II). Fig. 8 shows that conalbumin synthesis is induced with progesterone at the same rate as with estrogen, but ovalbumin is induced more rapidly and then plateaus at a level 25% lower than with estrogen alone. Progesterone stimulates magnum growth less than estrogen. A large portion of the wet weight increase observed with progesterone (from 70 to 390 mg, Table II), is caused by edema and cellular hypertrophy since magnum DNA content increases only 1.5-fold after 5 days compared to 5-fold with estrogen (6). Because progesterone has little effect on tubular gland cell cytodifferentiation (8), the induction of egg white protein synthesis must be occurring predominantly in previously existing tubular gland cells formed during primary stimulation. Furthermore, the similarity in the extent of egg white protein synthesis induced by progesterone, compared to that induced by estrogen, suggests that the time course of induction during secondary stimulation is not significantly influenced by the cytodifferentiation of new tubular gland cells.

**Combination of Testosterone and Progesterone**—This combination results in a slight inhibition of egg white protein synthesis compared to progesterone alone (Table II).

**Combination of Testosterone with Estrogen**—In contrast to above, combination of testosterone with estrogen has a marked synergistic effect on magnum growth and on the induction of ovomucoid synthesis, and to a lesser extent conalbumin synthesis, compared to estrogen alone (Table II). The synergistic effect of testosterone on growth is partly due to cellular hypertrophy and partly due to an increase in cell number since the DNA content does not increase proportionately with wet weight (Table III). The augmentation in conalbumin synthesis and content, as well as magnum growth, that is produced when testosterone is administered with estrogen has been noted previously under slightly different experimental conditions (1, 2, 9).

**Combination of Progesterone and Estrogen**—With this combination growth is inhibited, compared to estrogen alone, but the synthesis of ovomucoid and conalbumin is significantly enhanced, even more than with estrogen plus testosterone (Tables II and III). After 4 to 5 days of secondary stimulation with estrogen plus progesterone, conalbumin synthesis is almost double, and ovomucoid synthesis is 3 to 4 times greater than with estrogen stimulation alone. The time course of the induction of all four proteins is shown in Fig. 9. In these experiments the magnum

| Homones | Magnum weight | Relative rate of protein synthesis |
|---------|---------------|----------------------------------|
|         | mg/day        | OV | CON | MU | LY3 |
| E₂      | 0.90          | 47.0 | 9.6 | 2.2 | 2.1 |
| T₂      | 0.08          | 0.4 | 0.1 | 0.3 | 0.2 |
| P₄      | 0.39          | 37.2 | 8.8 | 1.5 | 2.2 |
| P₄+T₄  | 0.34          | 24.8 | 9.2 | 1.6 | 1.4 |
| E₂+P₂  | 2.18          | 42.7 | 11.4 | 6.6 | 1.6 |
| E₂+P₂  | 0.80          | 81.6 | 18.6 | 8.4 | 1.8 |
| Control | 0.07          | 0.3 | 0.6 | 0.2 | 0.2 |

*OV*, ovalbumin; *CON*, conalbumin (ovotransferrin); *MU*, ovomucoid; *LYS*, lysozyme.

**Fig. 9.** Comparison of the relative rate of specific egg white protein synthesis during secondary stimulation with estrogen or estrogen plus progesterone. Chicks (20 to 30 days withdrawn, 350 to 450 g, two to three per group) were given up to 5 days of secondary stimulation with estrogen (2 mg per day) or estrogen plus progesterone (2 mg each per day). At the indicated times chicks were killed and magnum explants incubated 21 hours with either \(^{14}C\)-amino acids (for estrogen-treated chicks, ○—○) or \(^{14}C\)-amino acids (estrogen plus progesterone-treated chicks, □—□); the explants were then pooled, homogenized, and centrifuged. The relative rate of protein synthesis was determined as in Fig. 3. In these experiments Triton X-100 was used during the washing of the immunoprecipitates reducing the anti-bovine serum albumin background to less than 0.1%. *OV*, ovalbumin; *CON*, conalbumin; *LYS*, lysozyme; *MU*, ovomucoid.
from estrogen-plus-progesterone-treated chicks were labeled with
4C-amino acids while magnum from estrogen-treated chicks
were labeled with 3H-amino acids; the immunoprecipitations
were performed with supernatants derived from the pooled tissues,
thus eliminating possible procedural differences. The data for
kinetics of ovalbumin, ovomucoid, and lysozyme induction are
all similar in that it takes 4 to 5 days to reach maximal induction
with either estrogen or estrogen plus progesterone. The induc-
tion of conalbumin synthesis is peculiar since maximal values of
10% of total protein synthesis are reached in 2 days with estro-
gen, and values of 20% are reached in 5 days with estrogen plus
progesterone; furthermore, the curves are not linear.

**Combinations of Estrogen, Progesterone, and Testosterone**—The
data of Table II suggest that administering some combination of
estrogen, progesterone, and testosterone, each at the appropriate
concentration, might produce relative rates of synthesis of each
egg white protein similar to those achieved naturally in laying
hens which have been reported (16) and are: ovalbumin, 64.2%;
conalbumin, 12.1%; ovomucoid, 8.6%; lysozyme, 1.5%.

Two series of experiments were tried: (a) a saturating dose of
estrogen (2 mg) with varying concentrations of progesterone, and
(b) saturating doses of estrogen and testosterone (2 mg) with
varying amounts of progesterone; in each case the hormones were
administered for 5 days as a secondary stimulation. Table III
shows that increasing the dose of progesterone augments ovom-
cucoid and conalbumin synthesis in both series of experiments.
With higher doses of progesterone, conalbumin synthesis becomes
greater than physiological. In addition, growth is progressively
inhibited with increasing progesterone concentration. As noted
above, testosterone has a synergistic effect on growth whether
measured as wet weight, DNA, or protein (Table III). The
combination of 2 mg each of estrogen and testosterone and 0.1
mg of progesterone appears to be the most physiological combi-
nation of hormones for inducing egg white protein synthesis in
chicks. After 5 days of secondary stimulation with this com-
bination, over 80% of the proteins being synthesized are secretory
proteins, and the magnum are almost 1% of the body weight,
values similar to those of actively laying hens.

**Table III**

| Hormones | Magnum wet weight | Magnum protein | Magnum DNA | Relative rate of protein synthesis |
|----------|------------------|----------------|-----------|-----------------------------------|
| mg/day   | g mg | mg | mg | % total |
| E₂       | 1.34 160 | 3.48 | 53.8 | 9.6 3.5 2.5 |
| E₂P₁      | 1.81 232 | 3.85 | 56.3 | 11.7 5.6 2.1 |
| E₂P₂      | 1.38 225 | 3.52 | 56.8 | 17.7 9.2 2.4 |
| E₂P₃      | 1.94 109 | 1.44 | 55.2 | 22.6 9.9 2.1 |
| E₂T₁      | 2.32 432 | 4.86 | 58.8 | 11.4 6.7 2.4 |
| E₂T₁P₁     | 2.93 404 | 4.55 | 58.9 | 13.8 8.9 1.8 |
| E₂T₁P₂     | 1.87 201 | 1.65 | 51.1 | 19.4 10.4 2.1 |
| E₂T₁P₃     | 1.35 151 | 1.46 | 53.3 | 22.4 11.0 2.2 |

*OV*, ovalbumin; *CON*, conalbumin (ovotransferrin); *MU*,
ovomucoid; *LYS*, lysozyme.

One method of estimating the half-life of mRNA is to stop
RNA synthesis and then measure the decay in the rate of specific
protein synthesis. The rates of degradation of several egg white
protein mRNAs have been measured in this way by pulse labeling
separate cultures of magnum tissue with radioactive amino acids
at various times after addition of actinomycin D (at 10 μg per
ml, a concentration which inhibits RNA synthesis greater than
98% (12)). The t₁/₂ of ovalbumin mRNA has been estimated in
magnum tissue from chicks receiving 5 days of primary stimula-
tion, 1 and 5 days secondary stimulation; in each case the t₁/₂ was
between 12 to 14 hours (12). Ovomucoid mRNA also has a t₁/₂
of about 14 hours, but conalbumin mRNA has a faster rate of
degradation with a t₁/₂ of 6 to 8 hours. The average mRNA t₁/₂
for nonsecretory proteins (total protein minus immunoprecipita-
ted egg white proteins) is even shorter, t₁/₂ of 4 to 5 hours (12).

To explore the mechanism by which estrogen plus progesterone
augments ovomucoid and conalbumin synthesis compared to es-
trogen alone, the relative rates of mRNA degradation were com-
pared after hormonal stimulation with the two treatments.

These experiments were conducted as described above, although
a double label technique was employed; tissue from estrogen-plus-
progesterone-treated chicks was labeled with 14C-amino acids
while tissue from estrogen-treated chicks was labeled with 3H-
amino acids. The results are plotted as percentage of total pro-
tein; thus after blocking RNA synthesis with actinomycin D,
those proteins with relatively long lived mRNAs will comprise a
progressively greater percent of the total protein synthesized, and
vice versa. For comparative purposes this seemingly indirect
method of measuring mRNA half-lives is simpler and more re-
liable than attempting to measure actual rates of protein syn-
thesis.

The results of two experiments, one conducted after 1 day of sec-
dary stimulation with estrogen or estrogen plus progesterone
stimulation (Fig. 10A) and the other after 3 days secondary
stimulation (Fig. 10B), are shown. In both experiments all the
secretory proteins measured become a larger percentage of the
total protein synthesized while nonsecretory proteins become a
smaller percentage. The actual slopes of the curves depend on
the relative amounts of mRNA of each half-life; thus, in the ex-
periment illustrated in Fig. 10A the slopes for all the secretory
proteins are greater than in Fig. 10B because there are more
mRNAs with short half-lives after 1 day than after 3 days of
secondary stimulation. However, within an experiment the
slope should be proportional to the mRNA half-lives. Ovalbu-
min and ovomucoid mRNAs have the longest half-lives, followed
by lysozyme, conalbumin, and nonsecretory protein mRNAs.
Comparison of the slopes of each specific protein after estrogen
or estrogen plus progesterone treatment in either experiment reveals
little difference in the rates of mRNA degradation. If anything,
there are slightly shorter mRNA half-lives for ovomucoid and
conalbumin after 3 days of estrogen plus progesterone treatment,
thus the increased synthesis of these proteins after this treatment
is not due to mRNA stabilization.

**Discussion**

**Increases in Specific Translatable mRNA Concentration**—As
a result of hormonal stimulation of immature chicks, egg white
protein synthesis rises from undetectable levels to 80 to 90% of
the total protein being synthesized in oviduct magnum (Tables
II and III). These increases in specific protein synthesis proba-

1 Palmiter, R. D. (1972) *J. Biol. Chem.* 247, in press.
were given either 1 day or 3 days secondary stimulation with estrogen (2 mg per day) or estrogen plus progesterone (2 mg per day). Magnum explants from estrogen or estrogen plus progesterone-treated chicks were cultured in Hanks containing 3II-amino acids (estrogen-treated chicks; e - e) or 14C-amino acids (estrogen plus progesterone-treated chicks, o - o). The magnum explants were then pooled and the relative rate of protein synthesis determined as in Fig. 9. Anti-bovine serum albumin backgrounds were less than 0.1%. N.S. (squares), nonsecretory protein, total protein minus sum of egg white proteins. OV, ovalbumin; CON, conalbumin; LYS, lysozyme; MU, ovomucoid.

Fig. 10. Comparison of estrogen and estrogen plus progesterone treatment on the relative rate of specific egg white protein synthesis in culture after inhibition of RNA synthesis with actinomycin D. Chicks (30 days withdrawn, 420 g, six per group) were given either 1 day (A) or 3 days (B) secondary stimulation with estrogen (2 mg per day) or estrogen plus progesterone (2 mg each per day). Magnum explants from estrogen or estrogen plus progesterone-treated chicks were cultured in Hanks’ containing actinomycin D (10 μg per ml), and pulsed for 1 hour with either 3H-amino acids (estrogen-treated chicks; □ - □) or 14C-amino acids (estrogen plus progesterone-treated chicks, ○ - ○). The magnum explants were then pooled and the relative rate of protein synthesis determined as in Fig. 9. Anti-bovine serum albumin backgrounds were less than 0.1%.

N.S., (squares), nonsecretory protein, total protein minus sum of egg white proteins. OV, ovalbumin; CON, conalbumin; LYS, lysozyme; MU, ovomucoid.

bly reflect corresponding changes in the translatable concentration of specific mRNAs. This has been shown for ovalbumin where the increase in its synthesis parallels the concentration of ovalbumin-synthesizing polysomes (16) and the extractable mRNA activity as measured in a cell-free protein-synthesizing system (19). If these findings can be generalized to include the other egg white proteins; they suggest that a major function of estrogen and progesterone in the magnum is to regulate specific mRNA concentrations which are, in turn, a function of rates of mRNA synthesis/activation and mRNA degradation.

Coordinate Induction of Egg White Protein mRNAs—The induction of egg white proteins appears coordinate when enough estrogen is administered to chicks to produce a maximal response, doses which presumably saturate all the specific binding sites. All the proteins are induced at the same time after secondary stimulation and for three of them, the relative rates of synthesis increase in a nearly linear fashion for 4 days and then begin to plateau thereby keeping their rates of synthesis in constant proportion (Fig. 9). The exception to this pattern, conalbumin, whose synthesis plateaus earlier, (Figs. 4, 6, 8, 9) can be explained by its shorter mRNA half-life. In culture, the absolute rate of conalbumin synthesis declines with a t1/2 of about 7 hours compared to 14 hours for ovalbumin, whether RNA synthesis is inhibited with actinomycin D or not. Also, the relative rate of conalbumin synthesis increases less than the other secretory proteins after actinomycin D treatment (Fig. 10). Theoretically, the time course of the change in mRNA concentration, which results from a change in mRNA synthesis/activation, should reflect the t1/2 of the mRNA, just as the approach of protein concentrations to new steady state levels after a change in the rate of protein synthesis depends on the rate of protein degradation (21), i.e. the shorter the t1/2, the faster the new steady state level should be reached. Differential translation of conalbumin mRNA is ruled out because of results indicating that after both 1 and 3 days of secondary stimulation the relative rate of conalbumin and ovalbumin polypeptide initiation and elongation stay in nearly constant proportion to each other.

Thus, after secondary stimulation with saturating doses of estrogen, and perhaps with other hormonal regimens as well, the working model is that there is essentially a step change in the rate of synthesis/activation of the mRNAs for each of the egg white proteins studied. There is a lag of about 5 hours, then these mRNAs enter the pool of translatable mRNAs at nearly constant rates for the next several days. But, because of its greater rate of degradation, conalbumin mRNA concentration plateaus sooner than the others.

Independent Regulation of Egg White Protein Synthesis—This model suggests that the induction of mRNA synthesis/activation of the various egg white proteins could be linked via some common regulatory factor. However, several experimental findings indicate that the model must be more complex since the synthesis/activation of the various mRNAs appear to be controlled independently.

If the model were correct, then one would predict that during withdrawal conalbumin synthesis would decline faster than ovalbumin synthesis because of its shorter t1/2, contrary to that observed in Fig. 4. This discrepancy suggested the experiments which showed that conalbumin synthesis is preferentially induced at low estrogen concentrations (Figs. 5 to 7). Thus, a likely reconciliation of these facts can be envisaged if the concentration of estrogen in the serum falls gradually during withdrawal and reaches the threshold for ovalbumin synthesis before reaching the threshold for conalbumin synthesis. The results illustrated in Fig. 7 support this contention. Another test would be to measure the decay in ovalbumin and conalbumin synthesis after treatment of chicks with a potent anti-estrogenic compound.

Other evidence for independent regulation of egg white mRNA synthesis/activation comes from experiments measuring induction with different steroids. For example, Fig. 8 shows that with progesterone treatment the ratio of ovalbumin to conalbumin synthesis during secondary stimulation is different from that with estrogen treatment. Also, with estrogen plus testosterone the induction of ovalbumin is not significantly altered whereas ovomucoid and conalbumin are induced to greater extents (Tables II and III). The comparison of estrogen treatment with estrogen plus progesterone treatment was studied most thor-
oughly because of the prominent effects of the latter treatment on ovomucoid and conalbumin synthesis (Fig. 9). Translational differences are an unlikely explanation because the rate of conalbumin polypeptide elongation and initiation remain in constant proportion to ovalbumin with both hormonal treatments, and the average rate of initiation and elongation for all proteins is similar with either treatment. Comparison of the two treatments reveals no significant differences in the rates of mRNA degradation for any of the egg white proteins (Fig. 10). Thus, differences in the rates of mRNA synthesis/activation seems to be the most likely explanation of increased ovomucoid and conalbumin synthesis when progesterone is present with estrogen.

The similarity in the time course of ovomucoid induction with either hormonal treatment (Fig. 9) is further evidence that there is an effect on mRNA synthesis/activation rather than degradation. With conalbumin, the time courses are not linear and they plateau at different times (Fig. 9) suggesting that the rate of conalbumin mRNA synthesis/activation may not be constant throughout secondary stimulation.

Relative Number of Egg White Protein mRNAs and Genes—By knowing the relative rate of synthesis for each of the proteins synthesized in a cell and the relative rates of mRNA degradation one can calculate (a) relative concentrations of each translatable mRNA and (b) relative gene frequencies. The main assumptions are that: (a) all genes are transcribed at the same rate, (b) all mRNAs are activated at the same rate, and (c) all mRNAs are translated at the same rate. There is some evidence to support the third assumption, but the others are unfounded. Table IV shows that the relative number of mRNAs is a function of the relative rate of synthesis and polypeptide length, whereas the relative gene frequency is the product of the relative rate of synthesis and degradation. If the assumptions are correct then for every lysozyme gene there are about 30 ovalbumin, 12 conalbumin, and 5 ovomucoid genes. If there are equal numbers of each of these genes, then there must be large differences in the rates of transcription or activation of the different mRNAs.

Steroid Receptors Although this paper does not deal directly with steroid receptors, it indicates that analysis of the interaction of these receptors with their target sites will be complex. Part of this complexity arises from the fact that there are at least two cell types in oviduct magnum which respond to these hormones, the tubular gland cells which synthesize the proteins studied here (see “Appendix”) and the goblet cells that synthesize avidin (22). Another aspect of the problem is that there are at least three different steroids that affect the magnun.

Both estrogen and progesterone receptors have been identified in chick oviduct and both have been found attached to chromatin (23, 24). O’Malley et al. have shown that there is specificity of the progesterone receptor for oviduct chromatin and this specificity appears to reside in the acidic proteins (25). The two receptors appear to be distinct proteins because of their different sedimentation properties in sucrose gradients. However, there is some competition for each receptor by the other active steroids (23, 26).

This study allows several predictions about the number and action of tubular gland cell steroid receptors. The facts that progesterone is inactive alone as a primary stimulation (8) and testosterone is always inactive alone indicates that there are at least three distinct receptors, one each for estrogen, progesterone, and testosterone. The progesterone receptors may be induced by estrogen since only after tubular gland cells cytodifferentiate do they respond to progesterone by synthesizing egg white proteins (26). There are probably multiple target sites for the receptors, rather than a single regulatory site which mediates many pleiotropic responses. The independent regulation of the induction of the egg white proteins is one piece of evidence, while the fact that as a secondary stimulation estrogen induces the synthesis of egg white proteins, ribosomes, and DNA whereas progesterone affects mainly the former, is another (6, 10). Furthermore, the difference in the threshold for conalbumin and ovomucoid induction suggests that the affinity of receptors for different targets may not be the same. The regulation of egg white synthesis is even more complicated by the suggestion that full activation of some receptor targets requires either receptors with both estrogen and progesterone bound, or both receptors bound at the same time, e.g. in the induction of ovomucoid. Finally, the testosterone receptor seems to act at a site distinct from either estrogen or progesterone receptors because it is synergistic with estrogen, but not progesterone, and inactive alone.

Contrast with Bacterial Operon—The regulation of egg white protein synthesis by steroids has certain gross similarities to the inducible enzyme systems in bacteria. In both cases the synthesis of a set of specific proteins is induced by low molecular weight effectors. Furthermore, both are dynamic control systems in that the effect is modulated by effector concentration rather than a simple “on-off” switch. However, in the inducible bacterial systems the effector binds to a repressor and thereby releases it from the DNA resulting in activation of gene transcription, whereas in the steroid-induced systems the effector binds to a receptor which presumably promotes mRNA synthesis/activation by binding to a target site, e.g. chromatin. Moreover, in either operon- or regulon-inducible systems there is a single regulator protein but in the oviduct there are several regulators involved, the different hormone receptors and their target sites.

Thus, the synthesis/activation of egg white protein mRNAs can be perturbed to alter the ratio of synthesis of the corresponding

| Table IV |
|-----------|
|          |
| Protein  |
| OV*      | CON | MU | LYS | N.S. |
| Relative rate of protein synthesis (% total)* | 60  | 12  | 10  | 2   | 16  |
| Relative rate of mRNA degradation* | 1   | 2   | 1   | ~1  | 3   |
| Relative number of mRNAs (% total) | 53  | 6   | 19  | 5   | 17  |
| Relative number of genes* | (60) | (24) | (10) | (2) | 30  | 12  | 5   | 1   |

* OV, ovalbumin; CON, conalbumin (ovotransferrin); MU, ovomucoid; LYS, lysozyme; N.S., nonsynthetic protein = total protein synthesis minus the sum of egg white protein synthesis.
* Approximate values determined in explants from laying hen (16) or E2T3P45E-treated chicks (Table III).
* Determined in culture with actinomycin D (Footnote 3 and Fig. 10).
* Determined by calculating the percentage total after dividing the relative rate of synthesis by polypeptide molecular weight (Table I). Average molecular weight of N.S. protein estimated at 35,000 (Footnote 3). Assumes that all mRNAs are translated at the same relative rate.
* Determined by multiplying the relative rate of synthesis by the relative rate of mRNA degradation (parentheses) and then dividing by the smallest number. Assumes all genes transcribed at the same relative rate, all mRNAs activated and translated at same rate.

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proteins but such perturbations do not occur in the bacterial controls. Another prominent difference is that in bacteria the effect of steroids may also promote cell division leading to more lysozyme, and conalbumin, is also appreciated.

but also stimulates the entire protein synthetic apparatus resulting in accumulation of tRNA (27), ribosomes (10, 28), endoplasmic reticulum (8, 12), translation factors, etc. Concomitantly steroids may also promote cell division leading to more competent cells capable of synthesizing the specific proteins (4–8).

Acknowledgements—I sincerely thank Dr. R. T. Schimke who provided both excellent research facilities and a stimulating atmosphere for conducting this research. I am grateful to Doctors R. Schimke, R. Cox, N. Carey, and N. Stebbing for constructive criticisms during the preparation of this manuscript. The help of P. LeTourneau and M. Holzer, who purified ovomucoid, lysozyme, and conalbumin, is also appreciated.

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APPENDIX

FLUORESCENT ANTIBODY LOCALIZATION OF OVALBUMIN, CONALBUMIN, OVOMUCOID, AND LYSOZYME IN CHICK OVIDUCT MAGNUM

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The morphology of the chick oviduct magnum after secondary stimulation with estrogen is shown in Fig. 1. The cells can be divided into three classes: (a) epithelial cells, including goblet and ciliated cells; (b) tubular gland cells with their prominent secretory granules; and (c) interstitial cells which include fibroblasts and various blood cells. Egg white proteins are presumed to be synthesized primarily in the tubular gland cells because of the conspicuous granules they contain after hormonal stimulation (1–6); however, one of the minor egg white proteins, avidin, has been localized in the epithelial cells (7).

Fluorescent antibody studies have localized ovalbumin (7, 8) and lysozyme (9) in the tubular gland cells but the localization of ovomucoid- and conalbumin-synthesizing cells was not known prior to this study. Of more importance, it was not known whether one or more of these proteins were synthesized in the same cell.

Fluorescent antibody localization of the egg white proteins was performed on magnun tissue from chicks given 1 day of secondary stimulation with estrogen plus progestosterone because this combination of hormones gives a maximal rate of induction of each of the proteins studied (see companion paper) and after 1 day the egg white proteins have not yet been secreted into the lumen of the glands where they could diffuse over the entire epithelium and thus confuse the fluorescent studies. Furthermore, secondary stimulation was deemed better than primary stimulation because the tubular gland cells are already present and hence do not have time to differentiate before synthesizing the egg white proteins (6, 9, 10). Also, most of the tubular gland cells lie below the surface epithelium, whereas during primary stimulation the differentiating tubular gland cells begin to synthesize the secretory proteins prior to their morphogenetic movements out of the surface epithelium (6).

The localization of ovalbumin and conalbumin in two adjacent 4-μm sections is shown in Fig. 2. Fig. 2a is a sketch of the sec-
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J. Biol. Chem. 1972, 247:6450-6461.

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