Interaction of Estrogen, Progesterone, and Testosterone in the Regulation of Protein Synthesis in Chick Oviduct*

Richard D. Palmiter

ABSTRACT: The effects of progesterone on protein synthesis (and polysomes) in the magnum portion of the estrogen-primed chick oviduct reported here are contrary to those described by Means, A. R., and O'Malley, B. W. (*Biochemistry 10*, 1570). The administration of progesterone for 1 or 2 days to chicks which had been treated with estrogen daily for 5–9 days results in: (1) an increase in the proportion of ribosomes which sediment in sucrose gradients as polysomes. The average size of the polysomes is unaffected; the major polysomal peak corresponds to aggregates of 10–14 ribosomes. (2) Small increases in the relative rate of ovalbumin and conalbumin synthesis; *i.e.*, these oviduct secretory proteins become a larger portion of the total protein being synthesized in the magnum. (3) Little change in the molecular weight distribution of the proteins being synthesized as measured by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. Therefore, progesterone appears to maintain protein synthesis in estrogen-primed chicks. The androgen, 2α-methyldihydrotestosterone, has a synergistic effect on oviduct magnum growth and differentiation when estrogen is present. This steroid affects polysomes and secretory protein synthesis in a manner similar to that observed when progesterone is administered to estrogen-primed chicks. However, after chicks have been withdrawn from estrogen for several weeks, testosterone does not stimulate the oviduct to synthesize the characteristic secretory proteins, contrary to the effect of either estrogen or progesterone.

The chick oviduct system provides a useful system for the study of steroid hormone regulation of cytodifferentiation and function of a specific cell type, the tubular gland cell which synthesizes the major egg-white proteins. The interactions of estrogen, progesterone, and testosterone are highly complex and include a variety of antagonistic and synergistic effects (Oka and Schimke, 1969a,b; Muller *et al.*, 1970; Palmiter *et al.*, 1970; Means and O'Malley, 1971).

Experiments from several laboratories indicate that progesterone treatment of estrogen-primed chicks maintains protein synthesis in general and the synthesis of the differentiated products, ovalbumin and lysozyme, in particular (Oka and Schimke 1969a,b; Korenman and O'Malley, 1968; Muller *et al.*, 1970). A recent report by Means and O'Malley (1971) has cast doubt on this interpretation; this paper attempts to clarify the discrepancy.

In addition, the studies of Oka and Schimke (1969a) concerning the interaction of estrogen and testosterone, have been extended to compare the effects of testosterone on estrogen-primed and withdrawn chicks with those of progesterone.

Methods

White Leghorn chicks (4–8-days old) were used in all studies. All hormones were dissolved in sesame oil (10 mg/ml) and injected daily into the lower leg muscles. 17β-Estradiol benzoate was a gift from Schering and 2α-methyldihydrotestosterone proprionate was a gift from Syntex. Progesterone was purchased from Nutritional Biochemicals.

The experimental methods have all been published and are referenced in the figure and table legends.

Results and Discussion

Effect of Progesterone on Estrogen-Primed Chicks. Previous studies (Oka and Schimke, 1969b) showed that 2- to 5-days progesterone treatment of 5d1°S$(E)_{1mg}$ chicks[1] results in approximately the same growth of the oviduct magnum and the same increase in RNA, DNA, and lysozyme concentration in magnum tissue when compared to chicks which continued to receive estrogen. All of these parameters were higher than in chicks receiving 5d1°S$(E)_{1mg}$ and then unstimulated (withdrawn) for the remaining time. These results indicate that progesterone is at least equivalent to estrogen with respect to promoting growth and the accumulation of differentiated product (lysozyme). In addition, the data of Korenman and O'Malley (1968) connote that progesterone treatment of 7d1°S$(DES)_{5mg}$ chicks results in small increases in oviduct weight, ovalbumin concentration, and a significant increase in [^{3}H]leucine incorporation into oviduct proteins when compared to chicks receiving the vehicle (sesame oil) only. These results are to be contrasted with the overall decrease in protein synthesis and the disaggregation of polysomes caused by progesterone in estrogen-primed chicks as reported by Means and O'Malley (1971). Since protein synthesis is thought to occur on polyribosomes (Noll, 1965; Warner *et al.*, 1963), the maintenance of growth and differentiated function in the presence of progesterone appears incompatible with the disaggregation of oviduct polysomes.

Most of the polysomes in estrogen-treated oviduct magnum tissue appear to be bound to the endoplasmic reticulum when observed with the electron microscope (Palmiter *et al.*, 1970; Kohler *et al.*, 1969). Many investigators have shown that detergents effectively release membrane-bound polysomes

* From the Department of Pharmacology, Stanford University, Stanford, California 94305. *Received June 18, 1971.* This research supported by American Cancer Society Grant No. P 427.

[1] The abbreviation should be read as follows: 5d1°S$(E)_{1mg}$ = 5 days of primary stimulation with 17β-estradiol benzoate at a dose of 1 mg/day. Other abbreviations are: DES, diethylstilbestrol; P, progesterone; T, 2α-methyldihydrotestosterone.

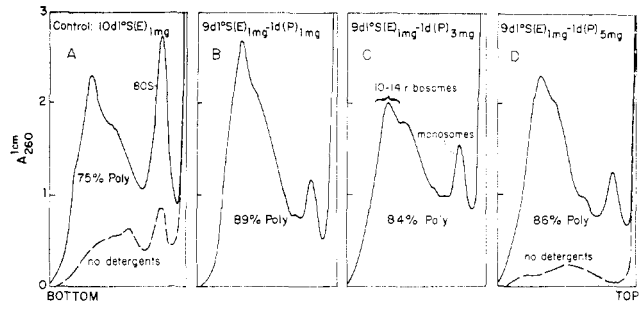

FIGURE 1: Polysome profiles from chick magnum. Polysomes were isolated and centrifuged as described in Table I. The measured absorbancy at 260 mμ was multiplied by 5 to correct for the 2-mm light path of the flow cell. The per cent of total ribosomes as polysomes (% poly) was calculated as before (Palmiter *et al.*, 1970).

(Wilson and Hoagland, 1965; Gerlinger *et al.*, 1969; Turkington and Riddle, 1970; Andrews and Tata, 1971). Means and O'Malley (1971) did not generally use detergents in their isolation procedures, and as a consequence may have isolated only the small fraction of ribosomes which were not bound to the membranes. When they added detergents to the supernatant *after* an initial centrifugation for 15 min at 14,000*g*, no effect on the recovery of ribosomes was observed; presumably because in tissues with extensive endoplasmic reticulum, like the oviduct, the bulk of the endoplasmic reticulum is pelleted under these conditions (Carey, 1966).

Table I shows the effect of detergents on the isolation of polysomes from 10d1°S(E)$_{1mg}$ chicks and from chicks treated with 5 mg of progesterone for the last day instead of estrogen. With the isolation procedure used here, the detergents (1% sodium deoxycholate and 1% Triton X-100) are added to the tissue homogenate *before* centrifugation; we have shown that with this procedure over 95% of the ribosomes are recovered (R. D. Palmiter)[2] and are undegraded (Palmiter *et al.*, 1970; R. D. Palmiter). The results show that without detergents the recovery of ribosomes from estrogen-treated chicks is about 28% of that when detergents are used. Moreover, if no detergents are used, only 9% of the ribosomes are extracted after progesterone treatment. Progesterone, therefore, appears to increase the proportion of ribosomes that are membrane bound. When no detergents are used, the recovery of ribosomes is about 2.4 μg/mg of tissue, which is within the range (1.2–1.7 μg of ribosomal protein/mg of tissue) reported by Means *et al.* (1971).

Figure 1 shows the sedimentation characteristics of the ribosomes isolated by both methods. When the total ribosome population is analyzed, by using detergents, progesterone has just the opposite effect on oviduct polysomes from that reported by Means and O'Malley (1971). Progesterone increases the proportion of ribosomes which sediment like polysomes; between 84 and 89% of the ribosomes appear as polysomes. The size distribution of the polysomes does not change significantly with any of the doses of progesterone used. The major A_{260} peak consists of polysomes containing 10–14 ribosomes, a size that is consistent with the average molecular weight of the proteins being synthesized in this tissue (38,000) (R. D. Palmiter).[2] Also, this is the predominant polysome size observed in planar sections of tubular gland cell endoplasmic reticulum (Palmiter *et al.*, 1970).

Figure 1 shows that 1 day of treatment with 1, 3, or 5 mg of progesterone all have approximately the same effect on polysome profiles. Figure 2 shows that progesterone treatment (1 mg) for 2 days has essentially the same effect as that observed after 1 day.

One interpretation of the increase in the proportion of ribosomes that sediment as polysomes after progesterone treatment could be that there is a selective loss of monosomes during the isolation of ribosomes from progesterone-treated chicks. To investigate this possibility the recovery of ribosomes was estimated by administering [^{3}H]uridine (100 μCi) to 7d1°S(E)$_{1mg}$ chicks. One day later half the chicks were stimulated with estrogen (1 mg) and the other half with progesterone (1 mg). After another day the polysomes were isolated

TABLE I[a]

Treatment	Magnum Wt (mg)	Ribosome Concentration: With Detergents μg/mg	mg/Oviduct	Without Detergents (μg/mg)	% "Free"
Control (estrogen)	560	8.6	4.8	2.4	28
Progesterone (1 mg)	770	7.4	5.7		
Progesterone (3 mg)	650	6.8	4.4		
Progesterone (5 mg)	530	6.8	3.6	0.6	9

[a] Effect of detergent treatment on ribosome isolation. Control chicks received 10d1°S(E)$_{1mg}$ while progesterone-treated chicks (2/group) received 9d1°S(E)$_{1mg}$ followed by 1-day stimulation with 1, 3, or 5 mg of progesterone. Both hormones were dissolved in sesame oil (10 mg/ml). Ribosomes were isolated using a slight modification of a previously published method (Palmiter *et al.*, 1970). Oviduct magnum tissue was homogenized in Tris buffer (25 mM Tris, 25 mM NaCl, and 5 mM $MgCl_2$) containing 0.14 M sucrose and 100 μg/ml of heparin, using seven strokes of a Dounce-type homogenizer. If detergents deoxycholate and Triton X-100 were used they were added from a 5% stock solution in distilled water to a final concentration of 1% for each, and the tissue was homogenized with two more strokes. The final concentration of tissue was always 100 mg (wet wt)/ml. The homogenate was centrifuged 4 min at 30,000g_{max}. (The addition of Triton X-100 prevents the formation of the viscous solution observed when only sodium deoxycholate is used and allows the homogenate to be centrifuged instead of filtered.) Supernatant (1 ml) was layered over a 11.4-ml gradient (0.5–1.5 M sucrose in the above buffer plus 40 μg/ml of heparin) and centrifuged for 1.6 hr at 283,000g_{max} in the SB-283 rotor (International Equipment Co.). The gradients were analyzed by passing them through a 2-mm flow cell connected to a Gilford recording spectrophotometer (see Figure 1). The ribosomal fractions were pooled and the absorbancy at 260 and 280 mμ measured. (The A_{260}:A_{280} ratio was between 1.68 and 1.73 when detergents were used and between 1.2 and 1.5 without detergents.) The concentration of ribosomes was determined from the relationship 1 A_{260} unit = 90 μg of ribosomes. "Free" ribosomes refers to the ratio of ribosome concentration isolated without detergents to that isolated with detergents.

[2] Submitted for publication.

TABLE II[a]

Treatment	Magnum Wt (mg)	Ribosome Concn (μg/ml)	% of Total: Oval-bumin Synthesis	% of Total: Conal-bumin Synthesis
Control	324	8.0	48.6	6.2
7d1 °S(E)$_{1mg}$			46.5	7.0
Progesterone				
5d1 °S(E)$_{1mg}$ 2d(P)$_{1mg}$	392	6.8	51.1	9.6
Testosterone				
5d1 °S(E)$_{1mg}$ 2d(T)$_{1mg}$	528	8.6	41.5	7.2

[a] Effect of progesterone or testosterone treatment of estrogen-primed chicks on specific protein synthesis. Chicks (3/group) were treated with progesterone or testosterone as indicated. Ribosome concentration was determined as in Table I. Pieces of oviduct magnum were cultured for 2 hr in Hanks salts with [^{3}H]amino acids (10 μCi/ml) for controls or [^{14}C]amino acids (1 μCi/ml) for progesterone- or testosterone-treated magnum. The tissues were homogenized with detergents (as in Table I) and the homogenate was centrifuged 1.5 hr at 105,000g_{max}. The per cent of the total acid-precipitable radioactivity which was also precipitable by antibodies specific for ovalbumin or conalbumin was determined as previously described (Palmiter *et al.*, 1971). All immunoprecipitations were performed in duplicate with different concentrations of antigen to be sure of antibody excess. The radioactivity in precipitates formed with anti-bovine serum albumin (less than 0.5% of total) has been subtracted. Total incorporation was approximately 20,000 cpm/mg wet wt for ^{3}H and 6000 cpm/mg for ^{14}C.

using standard procedures (as in Table I) and the per cent loss of acid-precipitable radioactivity in the 30,000g pellet (which is usually discarded) was determined. Since only 6% of the total radioactivity was in the pellet fraction with both treatments, not more than 6% of the total ribosomes could have been lost. Thus, this trivial explanation for the change in the polysome profile can be discarded. Alternatively, progesterone could create an imbalance (relative to estrogen-treated chicks) between the concentration of mRNA templates available for translation and the number of ribosomes. Either a relative increase in mRNA concentration or a decrease in ribosome concentration could account for the increased proportion of polysomes after progesterone treatment.

To investigate the proteins being synthesized in oviduct tissue after various hormone treatments, pieces of oviduct magnum were incubated in Hanks salt solution with radioactive amino acids (a procedure which initially mimics *in vivo* protein synthesis) (Palmiter *et al.*, 1971; R. D. Palmiter).[2] The relative rate of synthesis of the major oviduct secretory proteins (ovalbumin and conalbumin) was measured using immunological techniques which have been described in detail (Palmiter *et al.*, 1971). The results indicate that after progesterone treatment these secretory proteins account for a slightly larger percentage of the total protein being synthesized (Table II). Figure 3 illustrates the size distribution of

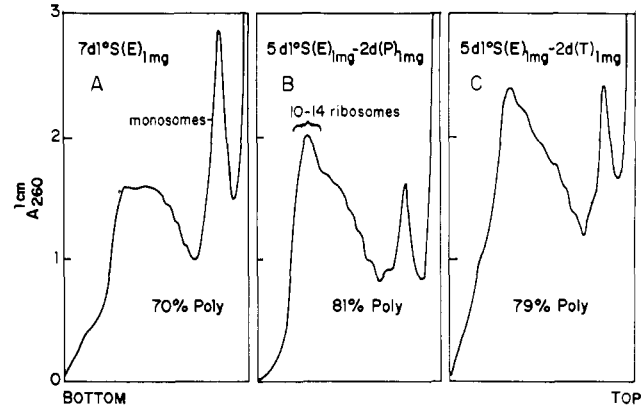

FIGURE 2: Polysome profiles from estrogen-primed chick magnum after 2 days of either progesterone or testosterone treatment. Same procedures as in Figure 1.

proteins synthesized in oviduct tissue after either 7d1 °S(E)$_{1mg}$ or 5d1 °S(E)$_{1mg}$ followed by 2 days with 1 mg of progesterone. The two major radioactivity peaks correspond to ovalbumin and conalbumin. Progesterone has little effect on the overall size distribution of the proteins being synthesized; this result is consistent with the negligible change in polysome size after progesterone treatment. Progesterone presumably induces avidin synthesis under these conditions (O'Malley, 1967; O'Malley and McGuire, 1968; Korenman and O'Malley, 1968). The subunit molecular weight of avidin is 18,000 (Green, 1964), hence it should migrate slightly slower (to the left) than

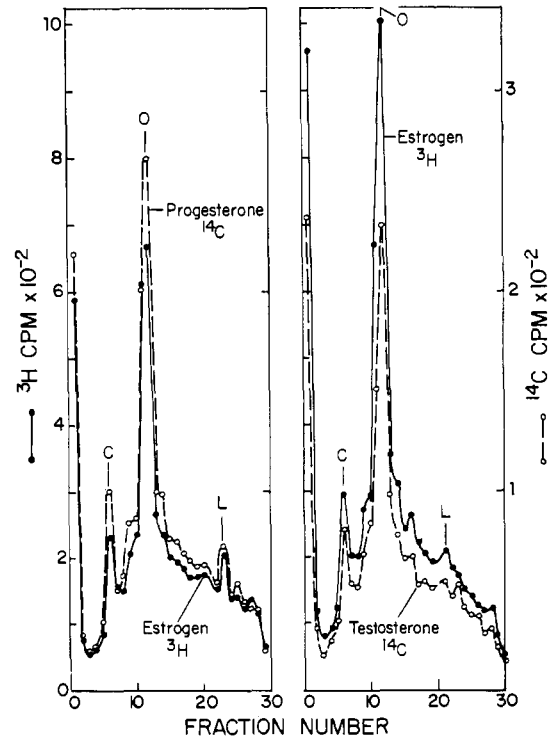

FIGURE 3: Migration of labeled magnum proteins on sodium dodecyl sulfate–polyacrylamide gels. Aliquots of the homogenates described in Table II were precipitated with 5% trichloroacetic acid, washed with trichloroacetic acid, dissolved, and electrophoresed on 10% SDS–polyacrylamide gels as previously described (Palmiter *et al.*, 1971). The migration of molecular weight markers is indicated by C, conalbumin (76,000); O, ovalbumin (42,000); L, lysozyme (14,000).

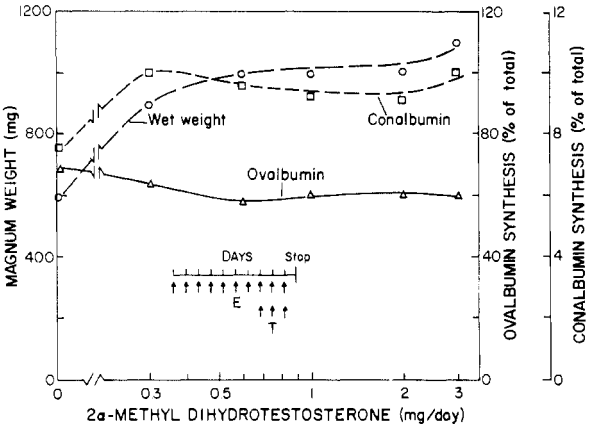

FIGURE 4: Dose response curve for testosterone. All chicks received 10d1°S(E)$_{1mg}$. During the last 3 days the indicated dose of testosterone was also administered to the chicks. Ovalbumin and conalbumin synthesis (expressed as per cent of total acid-precipitable radioactivity) was measured as in Table II.

TABLE III[a]

Treatment	Magnum Wt (mg)	% of Total Ovalbumin Synthesis	% of Total Conalbumin Synthesis
Control 21dW	68	<0.1	<0.1
Testosterone 1d2°S(T)$_{2mg}$	71	<0.1	<0.1
Estrogen 1d2°S(E)$_{2mg}$	253	16.3	7.5
Combination 1d2°S(E)$_{1mg}$(T)$_{1mg}$	261	13.7	5.9

[a] Effect of estrogen and/or testosterone treatment of withdrawn chicks on specific protein synthesis and growth. Chicks (2–3/group) which were withdrawn from estrogen stimulation for 20 days were injected with estrogen or testosterone as indicated. The magnum portion of the oviduct was removed 25 hr later and cultured for 2 hr in Hanks' medium with radioactive amino acids as in Table II. Total incorporation in acid-precipitable material was about 14,000 cpm/mg in the magnums of control and testosterone-treated chicks and about 6000 cpm/mg in the others. The relative rate of ovalbumin and conalbumin synthesis was determined as in Table II.

lysozyme (14,000) on sodium dodecyl sulfate–polyacrylamide gels. No radioactivity peak is observed in that position; however, since avidin accounts for such a small percentage of the total egg-white protein (0.1%, Warner, 1954), one might not expect this method to detect avidin synthesis.

Muller *et al.* (1970) measured the rate of protein synthesis *in vivo* when progesterone was given along with estrogen to estrogen-primed chicks. After taking into account the effect of progesterone on the specific activity of the amino acid pool, they concluded that progesterone has little or no effect on the rate of protein synthesis in the chick oviduct. Furthermore, Korenman and O'Malley (1968) measured the rate of ovalbumin synthesis *in vivo* using immunological techniques and found that the rate of isotope incorporation into this major protein was unaffected by progesterone treatment. These results along with the ones presented here, suggest that switching from estrogen treatment to progesterone treatment has a negligible effect on the rate of protein synthesis in the chick oviduct. The decreased rate of protein synthesis after progesterone treatment observed by Means and O'Malley (1971) may be attributed to the quality of the polysomes they used to measure the rate of protein synthesis with their cell-free protein-synthesizing system. If the polysomes isolated by them were either not representative of the total polysomal population and/or were partially degraded, then the rate of cell-free protein synthesis with those polysomes would not be expected to reflect the overall rate of protein synthesis *in vivo*. Furthermore, since cell-free protein-synthesizing systems are notoriously inefficient (rates of elongation are usually 100–1000 times slower than *in vivo*), it is difficult to draw meaningful conclusions about the regulation of protein synthesis using such systems.

The interaction of progesterone and estrogen in the regulation of the differentiation and function of chick oviduct magnum cells can be summarized as follows. (1) Administration of estrogen alone to immature chicks stimulates the differentiation of tubular gland cells and the synthesis of the major egg-white proteins: ovalbumin, conalbumin, and lysozyme (Kohler *et al.*, 1969; Oka and Schimke, 1969a,b; Palmiter *et al.*, 1970; Palmiter and Wrenn, 1971). (2) Administration of progesterone alone to immature chicks results in the differentiation of some oviduct epithelial cells (Cox and Sauerwein, 1970). Some of these cells may differentiate into goblet cells, although cell-specific proteins have not been identified in those cells. Tubular gland cells have not been observed to differentiate with progesterone alone (Cox and Sauerwein, 1970; Palmiter and Wrenn, 1971). (3) When progesterone is administered to immature chicks along with estrogen, there is initially a synergistic effect on the differentiation of tubular glands cells, but later the differentiation of these cells becomes abortive. The partially differentiated tubular gland cells do not migrate into the stroma as with estrogen treatment; instead, unusual flocculent secretory granules accumulate within the cells (Palmiter and Wrenn, 1971). (4) If estrogen is given for several days and then progesterone and estrogen are administered together, the two hormones have a synergistic effect on tubular gland cell function and growth (Oka and Schimke, 1969a,b; Muller *et al.*, 1970). If estrogen treatment is stopped and progesterone administered instead, progesterone appears to maintain the function of the tubular gland cells (Oka and Schimke, 1969a,b, and the subject of this paper). Moreover, goblet cells differentiate and commence to synthesize avidin when estrogen and progesterone are given together or progesterone replaces estrogen (O'Malley, 1967; Kohler *et al.*, 1968; O'Malley and McGuire, 1968). (5) Either estrogen or progesterone can induce the synthesis of the major egg-white proteins when given as a secondary stimulation to chicks withdrawn from estrogen stimulation (and hence not synthesizing egg-white proteins) (Oka and Schimke, 1969a,b; Palmiter *et al.*, 1970).

Effect of Testosterone (2α-Methyldihydrotestosterone) on Estrogen-Primed Chicks. (1) Administration of testosterone to immature chicks has little effect on oviduct growth compared to estrogen (Oka and Schimke, 1969a). (2) When testosterone (0.6–3.0 mg/day) is administered along with estrogen to 7d1°S(E)$_{1mg}$ chicks, there is a 60–70% increase in oviduct magnum wet weight (Figure 4). This result is consistent with the findings of Oka and Schimke (1969a). Testosterone also promotes growth when given instead of estrogen to estrogen-

primed chicks (Table II) but this result may depend upon low levels of estrogen remaining in the tissue. (3) Testosterone treatment of estrogen-primed chicks affects polysomes in a manner that resembles that observed with progesterone (Figure 2); the proportion of ribosomes that sediment like polysomes increases. (4) When testosterone is administered along with estrogen to estrogen-primed chicks the relative rate of ovalbumin synthesis decreases slightly (Table II), but considering the growth of the magnum, there is an increase in the total synthesis of ovalbumin by the oviduct. Oka and Schimke (1969a) observed an increase in the concentration of lysozyme when testosterone and estrogen were administered together. The relative rate of conalbumin synthesis usually increased with testosterone treatment (Figure 4). However, it is too early to say whether this hormone really has a differential effect on the synthesis of ovalbumin and conalbumin. (5) The molecular weight profile of the proteins being synthesized after testosterone treatment of estrogen-primed chicks appears similar to that when estrogen alone is given (Figure 3) indicating that testosterone does not induce the synthesis of any major proteins not already being synthesized. (6) Administration of testosterone (as a secondary stimulation) to estrogen-withdrawn chicks has no effect on growth or specific protein synthesis (Table III).

Thus, testosterone alone does not appear to promote tubular gland cell differentiation or function, but with estrogen there is some synergistic effect on growth and differentiation. Progesterone, in contrast, blocks normal estrogen-mediated cytodifferentiation, but can alone promote the function of already differentiated tubular gland cells. These results raise many questions about the number of steroid receptors in tubular gland cells and their mechanism of action.

Acknowledgment

I am grateful to Robert T. Schimke, in whose laboratory this work was performed, for both stimulating discussions and providing the facilities.

References

Andrews, T. M., and Tata, J. R. (1971), *Biochem. J. 121*, 683.
Carey, N. H. (1966), *Biochem. J. 100*, 242.
Cox, R. F., and Sauerwein, H. (1970), *Exp. Cell Res. 61*, 79.
Gerlinger, P., LeMeur, M. A., Beck, G., and Ebel, J. P. (1969), *Bull. Soc. Chim. Biol. 51*, 1157.
Green, N. M. (1964), *Biochem. J. 92*, 16c.
Kohler, P. O., Grimley, P. M., and O'Malley, B. W. (1968), *Science 160*, 86.
Kohler, P. O., Grimley, P. M., and O'Malley, B. W. (1969), *J. Cell Biol. 40*, 8.
Korenman, S. G., and O'Malley, B. W. (1968), *Endocrinology 82*, 11.
Means, A. R., Abrass, I. B., and O'Malley, B. W. (1971), *Biochemistry 10*, 1561.
Means, A. R., and O'Malley, B. W. (1971), *Biochemistry 10*, 1570.
Muller, K. R., Cox, R. F., and Carey, N. H. (1970), *Biochem. J. 20*, 337.
Noll, H. (1965), *Symp. Fundam. Cancer Res.*, 67.
Oka, T., and Schimke, R. T. (1969a), *J. Cell Biol. 41*, 816.
Oka, T., and Schimke, R. T. (1969b), *J. Cell Biol. 43*, 123.
O'Malley, B. W. (1967), *Biochemistry 6*, 2546.
O'Malley, B. W., and McGuire, W. L. (1968), *J. Clin. Invest. 47*, 654.
Palmiter, R. D., Christensen, A. K., and Schimke, R. T. (1970), *J. Biol. Chem. 245*, 833.
Palmiter, R. D., Oka, T., and Schimke, R. T. (1971), *J. Biol. Chem. 246*, 724.
Palmiter, R. D., and Wrenn, J. T. (1971), *J. Cell Biol. 50*, 598.
Turkington, R. W., and Riddle, M. (1970), *J. Biol. Chem. 245*, 5145.
Warner, J. R., Knopf, P. M., and Rich, A. (1963), *Proc. Nat. Acad. Sci. U. S. 49*, 122.
Warner, R. C. (1954), *Proteins 2*, 433.
Wilson, S. H., and Hoagland, M. B. (1965), *Proc. Nat. Acad. Sci. U. S. 54*, 600.