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Protein Biosynthesis on Chick Oviduct Polyribosomes. II. Regulation by Progesterone*

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ABSTRACT: Progesterone neither initiates oviduct cytodifferentiation nor elicits an overall increase in cellular hyperplasia. However, this hormone does specifically induce synthesis of the egg white protein avidin. The effect of progesterone on regulation of oviduct protein biosynthesis was investigated by examining synthesis and activity of polyribosomes following a single injection of this hormone to either unstimulated or estrogen-treated immature chicks. Progesterone stimulates polyribosomal protein synthesis within 24 hr following an injection to immature chicks. Moreover, analysis of the peptide products synthesized *in vitro* reveal differing patterns suggesting qualitative changes in protein populations produced by progestins.

These observations indicate that overall protein biosynthesis can be regulated by progesterone in the undifferentiated oviduct. This regulation may be mediated by earlier changes at the level of gene transcription resulting in synthesis of new messenger RNAs. On the other hand, progesterone administration to chicks previously treated with estrogen results in a disaggregation of polyribosomes and a decreased rate of ribosome synthesis. Twenty-four hours after injection, the ability of isolated polyribosomes to synthesize peptide *in vitro* is reduced by 40% and electrophoretic analysis of these newly synthesized peptides reveal qualitative differences. Furthermore, the radioactivity ratios of double-labeled gels suggest that progesterone may repress some nuclear genes normally active in the estrogen-treated oviduct as well as selectively activate a few previously restricted genes. These conclusions are compatible with the overall decrease in protein biosynthesis caused by progesterone in the differentiated oviduct coupled with the concomitant induction by this hormone of specific proteins such as avidin. Progesterone then appears to regulate protein biosynthesis in unstimulated or estrogen-differentiated oviducts in different manners. However, the primary effects must occur at the level of genetic transcription in both cases with at least one similar result—induction of avidin synthesis.

Progesterone specifically induces synthesis of the egg-white protein avidin within several hours following a single injection to DES-stimulated or unstimulated chicks (Korenman and O'Malley, 1968; O'Malley and McGuire, 1968a). Indeed the versatility of this response is remarkable since progesterone has also been shown to induce avidin synthesis *in vitro* in minces of oviduct tissue (O'Malley, 1967) and in monolayer cell culture (O'Malley and Kohler, 1967). In each case the rate of induction reaches a maximum between 12 and 24 hr after hormone administration.

Unlike the oviductal response to estrogen (Brant and Nalbandov, 1956; Kohler *et al.*, 1969; O'Malley *et al.*, 1969), progesterone neither initiates tissue differentiation and growth nor causes an overall increase in protein biosynthesis (O'Malley and McGuire, 1968a; O'Malley *et al.*, 1969, 1971). In fact, the effect of progesterone on protein biosynthesis is complex since administration of this steroid to chicks previously treated with DES results in a diminished rate of total protein biosynthesis (O'Malley *et al.*, 1971) whereas injection into unstimulated (O'Malley and McGuire, 1968a, O'Malley *et al.*, 1971) or estrogen-withdrawn (Palmiter *et al.*, 1970) animals brings about an increase in this parameter.

We have recently reported the interaction of progesterone with a specific cytoplasmic protein which may represent the initial biochemical response of the oviduct to this steroid (O'Malley *et al.*, 1970, 1971). Subsequent to this initial interaction the bulk of the evidence presently available points towards early primary effects upon nuclear transcription (O'Malley *et al.*, 1969, 1971; O'Malley and McGuire, 1968b, 1969). In order to provide evidence concerning the cyto-

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TABLE I: Polysomal Protein Recovered from Oviducts of DES-Treated Chicks at Various Times Following a Single Injection of Progesterone.[a]

Time after Progesterone (hr)	Polysomal Protein (μg/mg of Oviduct)	(μg/oviduct)
0	1.5	842.5
6	1.5	838.6
12	1.3	705.9
24	0.5	308.3
48	0.5	297.5

[a] Progesterone (5 mg) was administered as a single injection for the times indicated. Chicks had been pretreated with DES for 10 days. Polyribosomes were isolated from oviducts as previously described (Means *et al.*, 1969; Means *et al.*, 1971). Values represent the mean of 6 experiments.

TABLE II: Effect of Progesterone upon Polysomal Protein Recovered from Oviduct of Unstimulated or DES-Withdrawn Chicks.[a]

Chicks	Time after Progesterone (hr)	Polysomal Protein (μg/mg of Oviduct)	(μg/oviduct)
Unstimulated	0	0.6	12.3
	24	0.6	12.2
DES-Withdrawn	0	0.1	37.4
	24	0.1	45.2

[a] Progesterone was administered as a single subcutaneous injection 24 hr before killing the chicks. Unstimulated are those chicks which had received no hormone prior to progesterone, whereas the DES-withdrawn group received daily injections of DES for 10 days. Estrogen was then discontinued for 16 days immediately prior to the injection of progesterone. Polyribosomes were isolated as previously described (Means *et al.*, 1969; Means *et al.*, 1971). Values represent the mean of 6 determinations.

plasmic events following the entry of progesterone into the nucleus but prior to the appearance of avidin, we have isolated and characterized the polyribosomes from chick oviduct (Means *et al.*, 1971) and examined profiles and incorporation activity of particles isolated from unstimulated and hormone-treated oviducts.

The data to be presented will demonstrate that progesterone can regulate protein biosynthesis by oviduct polysomes from unstimulated or DES-treated chicks. Moreover, this regulation will be shown to involve alteration in polyribosome synthesis and/or aggregation as well as incorporation activity. Furthermore, we will demonstrate that this steroid results in the synthesis of qualitatively different peptides by oviduct polyribosomes *in vitro* following its administration to immature chicks. Finally, evidence will be presented that progesterone results in a positive regulation of oviduct protein biosynthesis when administered to unstimulated chicks. On the other hand, progesterone will be shown to regulate protein biosynthesis in differentiated oviduct *via* an antagonism of estrogen. This antagonistic effect is suggested to occur at the transcriptional level since estrogen-stimulated messenger RNAs and subsequent peptide synthesis are suppressed by progesterone.

Materials and Methods

Materials. Progesterone in peanut oil (50 mg/ml) was obtained from Eli Lilly and Co. Sources of all other materials have been described in a previous publication (Means *et al.*, 1971).

Methods. Progesterone (5 mg) was administered to immature unstimulated or DES-pretreated chicks as a single subcutaneous injection. All other methods have been previously described (Means *et al.*, 1969; Means *et al.*, 1971).

Results

Effect of Progesterone upon Polyribosomes Recoverable from Chick Oviduct. The effect of a single injection of progesterone to chicks previously treated with DES for 10 days upon oviductal content of polyribosomes is shown in Table I. No effect is observed for the first 6 hr and any effect is questionable at 12 hr. However, 24 hr following progesterone, a decrease is noted in polyribosomal protein whether expressed as micrograms per milligram of oviduct or micrograms per oviduct. No further decrease is demonstrable after an additional 24 hr (*i.e.*, 48 hr).

When progesterone is administered to unstimulated chicks (Table II) no detectable change occurs in the amount of polyribosomal protein recoverable from oviduct 24 hr following a single injection. Likewise no effect of progesterone is observed on this criteria when administered to chicks which had been treated for 10 days with DES and then untreated for an additional 16 days (Table II).

Distribution of Oviduct Ribosomes 24 Hr Following Progesterone Administration. Figure 1 illustrates the distribution

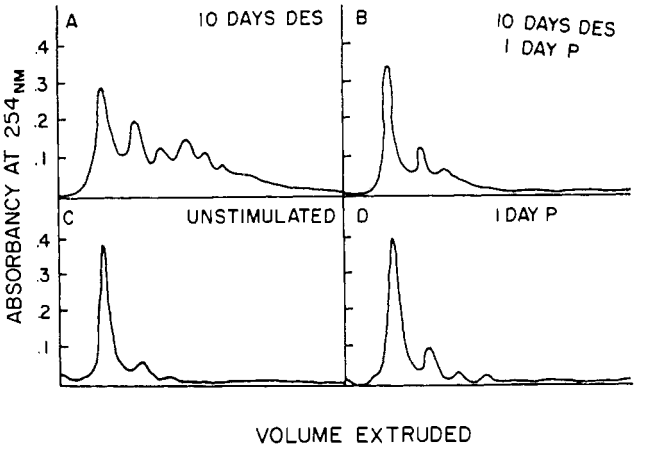

FIGURE 1: Sucrose gradient profiles of polyribosomes isolated from chick oviduct. Ten A_{254} units was applied to each 27-ml gradient (0.3 to 1.0 M). Centrifugation was continued for 2 hr at 25,000 rpm. Gradients were fractionated using an ISCO Model D density gradient fractionator. Direction of sedimentation was from left to right and the first peak in each panel represents single ribosomes: (A) 10 days DES; (B) 10 days DES plus 1 day progesterone; (C) unstimulated; (D) unstimulated plus 1 day progesterone.

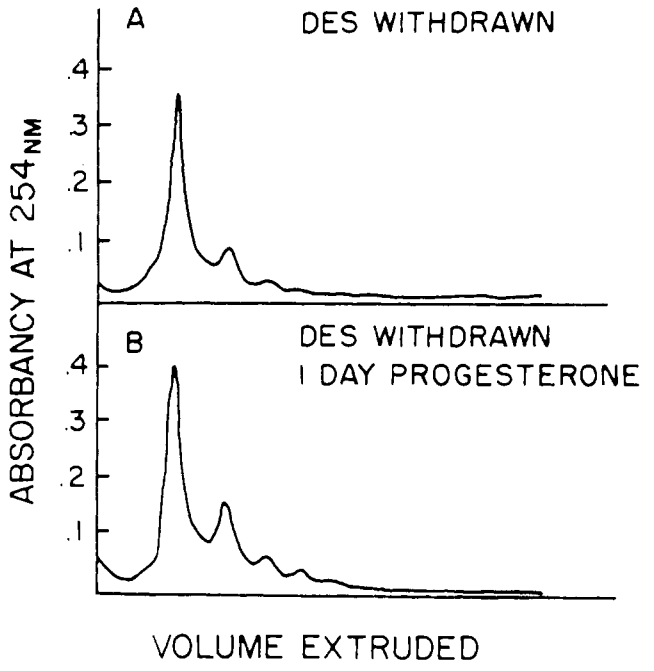

FIGURE 2: Sucrose gradient profiles of polyribosomes isolated from chick oviduct: (A) 10 days DES then hormone discontinued for 16 days; (B) 10 days DES then discontinuation of estrogen treatment for 16 days followed by a single injection of progesterone. For details of analysis, see legend to Figure 1.

of isolated oviduct polyribosomes on sucrose gradients. In each case direction of sedimentation is from left to right; the first peak from the left is representative of single ribosomes. Several peaks of absorbancy at 254 nm are observed in the profile of polyribosomes from 10-day DES-treated chicks (Figure 1A). A single injection of progesterone to 10-day DES-treated chicks results in a striking disaggregation of polyribosomes into smaller particles within 24 hr of a single injection (Figure 1B). Thus, few particles containing more than 3 ribosomes are demonstrable in this case.

Polyribosome profiles of unstimulated chick oviduct reveal a predominance of single ribosomes (Figure 1C). One day following progesterone administration to unstimulated chicks, little change is noted although the polyribosome profile appears to contain a slightly larger population of aggregates (Figure 1D).

The polyribosome pattern seen in DES-treated chicks subsequently withdrawn from estrogen is shown in Figure 2A. It can be seen to contain predominantly monomers and is remarkably similar to profiles from unstimulated and DES plus progesterone-treated chicks (compare with Figure 1B and C). Again a single injection of progesterone to DES-withdrawn chicks appears to result, to a very small degree, in the assembly of polyribosomes (Figure 2B).

TABLE III: Protein Synthesis by Isolated Oviduct Polysomes *in Vitro* at Various Times Following a Single Injection to DES-Treated Chicks.[a]

Time after Progesterone (hr)	Specific Activity (pmoles of [^{14}C]Val/mg of Ribosomal Protein)
0	34.9
6	35.0
12	33.5
24	21.6
48	18.8

[a] All chicks had received DES for 10 days prior to progesterone administration. Progesterone was given as a single injection for the times indicated. Polyribosomes were then isolated from oviducts and were incubated in a cell-free system (400 μg/tube) for 30 min at 37° (Means *et al.*, 1969; Means *et al.*, 1971). Values are the mean of 6 experiments and assays were performed in triplicate. One pmole of [^{14}C]valine is equivalent to 438 dpm in the acid-precipitable material. Counting efficiency was 85%.

TABLE IV: Protein Synthesis by Isolated Oviduct Polysomes *in Vitro* One Day Following a Single Injection of Progesterone to Unstimulated or DES-Withdrawn Chicks.[a]

Chicks	Time after Progesterone (hr)	Specific Activity (pmoles of [^{14}C]Val/mg of Ribosomal Protein)
Unstimulated	0	19.4
	24	32.2
DES-Withdrawn	0	18.3
	24	37.7

[a] Details of hormone treatment and experimental protocol are in the footnote to Table III. DES-withdrawn chicks were injected with estrogen daily for 10 days; injections were then discontinued for the subsequent 16 days.

Polyribosomal Protein Biosynthesis. EFFECTS OF PROGESTERONE. Administration of progesterone to DES-treated chicks causes little if any effect upon the ability of isolated polyribosomes to incorporate [^{14}C]valine into peptide (Table III) during the first 12 hr following injection. However, a decline in activity is demonstrated between 12 and 24 hr. By 48 hr following progesterone injection, protein biosynthesis is only approximately 50% of that observed by polyribosomes from the DES-treated control chicks.

Table IV illustrates that opposite results are obtained when progesterone is administered to either unstimulated or DES-withdrawn chicks. That is, a single injection of progesterone to unstimulated chicks causes within 1 day a substantial increase in the protein-synthesizing ability of isolated oviduct polyribosomes. Similarly a twofold stimulation is noted 1 day following progesterone injection to DES-withdrawn chicks.

Qualitative Analysis of Peptides Synthesized in Vitro. We have previously demonstrated that administration of progesterone to DES-treated chicks causes no changes in the soluble proteins detectable upon polyacrylamide gel electrophoresis of a 105,000*g* oviduct supernatant fluid (O'Malley *et al.*, 1970, 1971). Since this technique exhibits only limited sensitivity we decided to investigate the peptide products synthesized by oviduct polyribosomes *in vitro* by the more

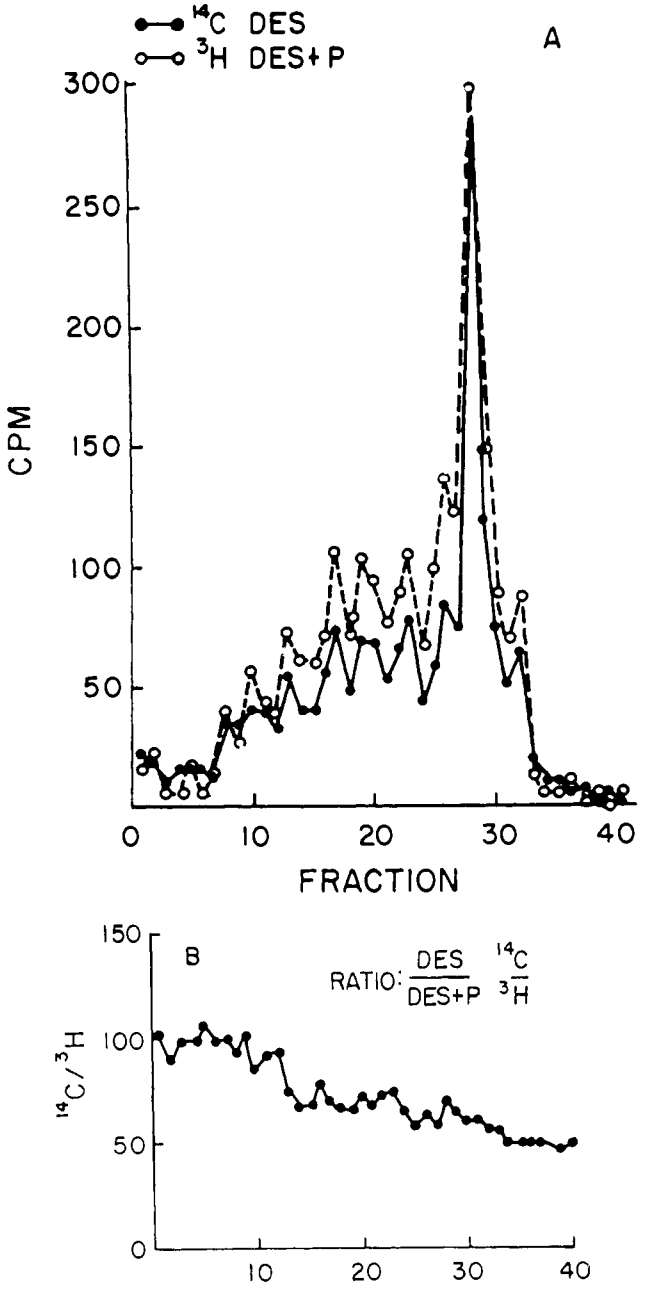

FIGURE 3: (A) Polyacrylamide gel electrophoretic analysis of peptides synthesized by oviduct polyribosomes *in vitro* at 10 days DES (●—●) and 10 days DES plus 1 day progesterone (○—○). Peptides were double labeled as follows: polyribosomes from 10 day DES chicks were incubated with [^{14}C]valine and those from 10 day DES plus 1 day progesterone with [^{3}H]valine. Following incubation for 45 min, samples were pooled and carried throughout remaining procedures together (Means *et al.*, 1971). Gels were fractionated and radioactivity of each fraction determined under optimal conditions for double labeling (Means *et al.*, 1971). Under these conditions of gel electrophoresis authentic advidin standard migrates to fraction 12, whereas major bands of ovalbumin and lysozyme correspond to fractions 16, 20, 22, and 25. (B) Ratio of ^{14}C/^{3}H cpm of the electrophoretogram shown in Figure 3A.

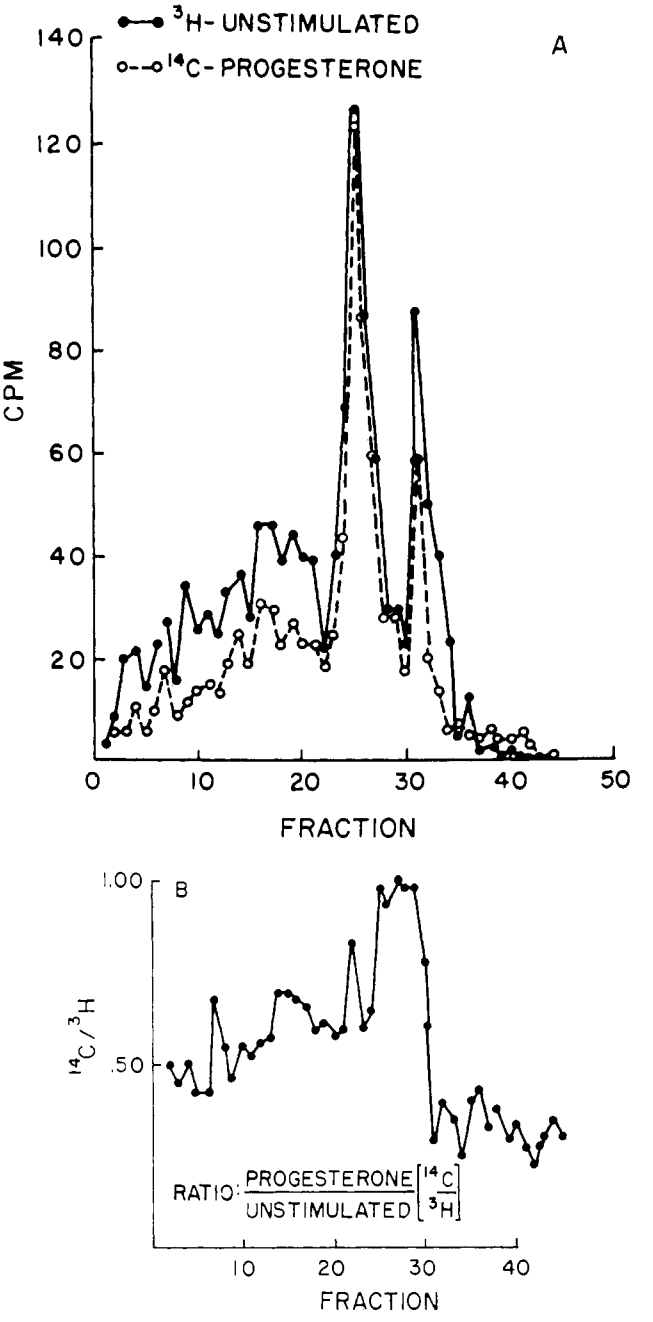

FIGURE 4: (A) Electrophoretogram of peptides synthesized by polyribosmes *in vitro* isolated from oviducts of unstimulated (●—●) or unstimulated plus 1 day progesterone (○—○) chicks. Details are in legend to Figure 3 (see also Means *et al.*, 1971); (B) Ratio of ^{14}C/^{3}H cpm of the electrophoretogram shown in Figure 4A.

definitive method of double labeling newly synthesized peptides. Thus polyribosomes were isolated from oviducts of chicks treated for 11 days with DES or for 11 days DES plus 1 day progesterone. These preparations were incubated as previously described (O'Malley *et al.*, 1970, 1971; Means *et al.*, 1971) where [^{14}C]valine was present as the radioactive amino acid for DES polyribosomes and [^{3}H]valine for particles from DES and progesterone-treated chicks. Peptides resulting from this double-label experiment were then analyzed by polyacrylamide gel electrophoresis. The electrophoretogram of such an experiment is presented as Figure 3A. It can be seen that no major differences are readily observable in the radioactivity tracings. When the radioactivity is expressed as the ratio of ^{14}C/^{3}H and plotted for each gel fraction, a constant ratio indicates that similar peptides are present in the products synthesized by both groups of polyribosomes (Means *et al.*, 1971). Figure 3B shows this type of plot for the data presented in Figure 3A. It is apparent that the ratio of ^{14}C/^{3}H approaches unity in the first 12

TABLE V: Effect Of Progesterone On Oviduct Ribonuclease.[a]

Treatment	Progesterone (24 hr, *in vivo*)	Ribonuclease (μg/mg of Protein) $-HgCl_2$	Ribonuclease (μg/mg of Protein) $+HgCl_2$
Unstimulated	−	2.7×10^{-7}	4.5×10^{-7}
	+	5.5×10^{-8}	4.4×10^{-7}
7 Days DES	−	1.6×10^{-7}	2.7×10^{-7}
	+	3.0×10^{-8}	2.2×10^{-7}
20 Days DES	−	2.9×10^{-8}	1.8×10^{-7}
	+	5.4×10^{-8}	1.6×10^{-7}

[a] Ribonuclease activity was measured in a 105,000*g* supernatant fluid of oviduct tissue by the method of Schrader and Greenman (1971). Data are expressed as microgram equivalents of pancreatic ribonuclease standard per milligram of oviduct-soluble protein. When present $HgCl_2$ was added to the assay at a final concentration of 6 μM. Data represent the mean value of 3 experiments with each assay performed in triplicate.

fractions, 0.75 in fractions 13–24, and 0.5–0.6 from 24 to 33. The usual ratio approaches 0.75 when no changes occur (Means *et al.*, 1971). Therefore one may assume that an increase toward unity suggests a depression by progesterone of synthesis of these peptides whereas a decrease from the average value suggests stimulation of synthesis. The changes seen in Figure 3 are indeed small. However, when the isotopes were reversed (*i.e.*, ^{14}C for DES-treated and ^{3}H for DES plus P) similar changes in ratio were also observed (unpublished results) thus lending further credence to the differences shown.

Experiments were performed similarly with polyribosomes from unstimulated chicks and chicks which had received a single injection of progesterone 24 hr before killing. In this case [^{3}H]valine was utilized to label peptides synthesized by unstimulated polyribosomes whereas those from progesterone-treated chicks were incubated with [^{14}C]valine. Electrophoretic analysis resulted in the radioactivity patterns shown in Figure 4A. A plot of the $^{14}C/^{3}H$ ratio reveals an increase in $^{14}C/^{3}H$ in fractions 23–31 to levels very close to 1.0 (Figure 4B). Again these data suggest an increased synthesis of peptides which occupy fractions 23–31 on the electrophoretogram. On the other hand, only slight changes appear in the first 23 gel fractions.

Effect of Progesterone on Oviductal Ribonuclease. Ribonuclease was measured by the procedure of Schrader and Greenman (1971) which measures the release of the dye thionine from high molecular weight RNA. Furthermore, enzyme activity was measured in the absence or presence of $HgCl_2$ (6 μM). A potent intracellular inhibitor of ribonuclease has been reported to be present in several tissues (Schrader and Greenman, 1971; Roth, 1958; Wilson and Hoagland, 1965) and the inhibitory influence of this compound is abolished by presence in the assay of $HgCl_2$ (Roth, 1958; Schrader and Greenman, 1971). Table V shows the effect of a single injection of progesterone upon ribonuclease levels in oviducts at different stages of DES-mediated differentiation. Data presented were obtained from a 105,000*g* supernatant fluid and are expressed as micrograms of ribonuclease per milligram of soluble protein. It can be seen that progesterone neither brings about an increase in ribonuclease levels in oviducts of unstimulated chicks nor in chicks treated for 7 or 20 days with estrogen. In fact, oviductal ribonuclease activity appears to decrease following progesterone administration. Moreover RNase was measured in crude homogenates, postmitochondrial supernatant fractions, and isolated polyribosomes from the same tissue samples. Again no detectable increases were observed due to progesterone administration.

Discussion

We have demonstrated that progesterone regulates protein biosynthesis by oviduct polyribosomes in both unstimulated and DES-stimulated chicks. Moreover, the direction of this regulatory influence depends upon the state of differentiation of the oviduct. On one hand, progesterone enhances protein biosynthesis in unstimulated chicks while, on the other hand, this steroid inhibits overall protein biosynthesis in oviducts of chicks previously treated with DES. These changes in protein biosynthesis are reflected by qualitative differences in the peptides synthesized *in vitro* by oviduct polyribosomes.

Progesterone has been reported to act as an estrogen antagonist in the oviduct system (Oka and Schimke, 1969a,b). Our data would support these observations since synthesis and activity of estrogen-treated oviduct polyribosomes are inhibited within 24 hr following a single injection of progesterone (Tables I and III). However, it will be seen from Table I that there may be a decrease in oviduct content of polyribosomes 24 hr following progesterone injection. It should be pointed out that our isolation procedure selects for mRNA-bound ribosomes and, in fact, the *total* microsomal protein concentration remains unchanged (*i.e.*, 2.56 mg/g of oviduct for DES *vs.* 2.53 mg/g of oviduct for DES plus P). Thus, these apparent discrepancies may be explained by the fact that progesterone also causes a disaggregation of polyribosomes into ribosomes (Figure 1). The population of peptides synthesized by polyribosomes isolated from oviduct following administration of progesterone to DES-primed chicks appears to be qualitatively different from polyribosomes from chicks treated with estrogen only (Figure 3). Although the overall differences may be small, the ratios suggest that progesterone may repress some genes normally active in the DES-treated chicks, on the one hand, but may also activate a relatively few previously restricted genes, on the other hand. This suggestion would be consistent with an overall depression of cell protein biosynthesis with concomitant induction of one or more specific proteins (O'Malley, 1967; O'Malley and Kohler, 1967; Korenman and O'Malley, 1968; O'Malley and McGuire, 1968a).

We have previously demonstrated that administration of progesterone to DES-treated chicks produce alterations in nuclear rapidly labeled RNA synthesis (O'Malley and McGuire, 1968a) and RNA polymerase activity (McGuire and O'Malley, 1968). Chromatin template capacity is also varied by progesterone (O'Malley *et al.*, 1969, 1971). A characteristic feature of these nuclear events is that an inhibition invariably precedes an increase. These data coupled with the present studies on cytoplasmic protein biosynthesis would raise the possibility that progesterone shuts down some previously functional nuclear genes and then activates others allowing synthesis of new messenger RNA molecules which are subsequently transported to, and translated in, the cytoplasm. Progesterone induces new hybridizable species of oviduct nuclear RNA (O'Malley and McGuire, 1968b,c,

1971) in DES-treated chicks which are paralleled temporally by the qualitative changes in peptide populations illustrated in the present studies (Figure 3). Indeed, progesterone administration to DES-treated chicks results in a greatly diminished synthesis of ovalbumin and lysozyme by tubular gland cells (Oka and Schimke, 1969a,b) but specifically induces avidin synthesis by goblet cells (Korenman and O'Malley, 1967; Kohler, *et al.*, 1968). Again these data suggest an effect of progesterone upon the nucleus to promote both selective gene repression and activation.

Alternatively the disaggregation of oviduct polyribosomes following administration of progesterone to DES-treated chicks could conceivably be due to an activation of ribonuclease by this hormone. Clearly, however, this is not the case since the data presented in Table V demonstrate that progesterone does not increase activity or amount of oviductal ribonuclease at several stages during estrogen-mediated tissue differentiation. Therefore the most reasonable explanation for the progesterone-mediated decrease in protein synthesis would be a lack of messenger RNA necessary for formation of functional polyribosomes.

The effect of progesterone on the undifferentiated oviduct is considerably different from the effect on tissue undergoing DES-mediated cytodifferentiation. On the other hand, progesterone results in both cases in the same specific end point—the induction of avidin synthesis (O'Malley, 1967; O'Malley and McGuire, 1968a; O'Malley and Kohler, 1967; O'Malley *et al.*, 1969, 1971). We have demonstrated that progesterone results in an overall increase in polyribosomal protein biosynthesis in the unstimulated oviduct (Table IV). On the other hand, no demonstrable increase in ribosome mass occurs within 24 hr following progesterone administration (Table II). These data indicate that the rate-limiting factor for protein biosynthesis in the unstimulated oviduct may be availability of messenger RNA. Support for a gene-activation hypothesis comes from our previous findings that progesterone induces new species of hybridizable RNA in oviduct nuclei prior to induction of avidin synthesis (O'Malley and McGuire, 1968b,c, 1969). Furthermore, administration of progesterone *in vivo* appears to induce synthesis by isolated oviduct polyribosomes of some qualitatively different peptides (Figure 4), suggesting the presence of new messenger RNAs. Alternatively progesterone may differentially effect the rate of synthesis of various peptides. However, pretreatment of chicks with actinomycin D prevents the assembly of ribosomes and hormone-mediated increase in protein biosynthesis by oviduct polyribosomes (Means and O'Malley, unpublished observations).

Assembly of polyribosomes from preexisting monomers have been demonstrated previously for hormone-sensitive tissues. Cohen and Stastny (1968) first demonstrated this control mechanism in studies on regulation of protein biosynthesis in chick embryo epidermis by epidermal growth factor (EGF). These investigators showed that polyribosomes were assembled from preexisting monomers by prelabeling ribosomal RNA with [^{14}C]uridine, withdrawing isotope for a period of time, and then adding EGF for a 1-hr period. This hormone caused a decrease in monomers and concomitant increase in polyribosomes with a corresponding decrease in [^{14}C]uridine from single ribosomes and its appearance in polyribosomes. Again experiments identical with those of Cohen and Stastny (1968) have been reported recently by Palmiter *et al.* (1970) using the chick oviduct system. In this case DES-treated chicks were withdrawn from estrogen for a period of time which resulted in a disaggregation of polyribosomes. Readministration of either DES or progesterone caused a reassembly of polyribosomes from the previously existing monomers.

Data presented in the present communication would be compatible with the experiments of Palmitter *et al.* (1970) concerning regulation of oviduct polyribosome formation by progesterone in the estrogen-withdrawn chick. Thus, it was demonstrated that DES-withdrawal resulted in polyribosome breakdown (Figures 1 and 2) and progesterone stimulates protein biosynthesis by polyribosomes isolated from oviducts of DES-withdrawn chicks within 24 hr after a single injection (Table IV) in the absence of effect upon ribosome mass (Table II).

Progesterone, therefore, stimulates protein biosynthesis in oviducts from both unstimulated and estrogen-withdrawn chicks. These actions, however, are different in several ways. The unstimulated oviduct is undifferentiated and no differentiation occurs as a result of progesterone administration (O'Malley and McGuire, 1968a; Kohler *et al.*, 1969; O'Malley *et al.*, 1969). Therefore, this steroid must be able to change the function of the primitive mucosal cells which is culminated by the appearance of a new population of proteins, one of which is avidin (O'Malley, 1967; O'Malley and Kohler, 1967; O'Malley and McGuire, 1968a; Kohler *et al.*, 1969; O'Malley *et al.*, 1971). On the other hand, although withdrawal of chicks from DES results in cessation of ovalbumin and lysozyme synthesis, the differentiated state of the oviduct (morphological) remains unaltered (O'Malley *et al.*, 1969, 1971; McGuire and O'Malley, 1968). Administration of progesterone now not only results in avidin induction in goblet cells (O'Malley *et al.*, 1969; Kohler *et al.*, 1968), but also stimulates gland cell synthesis of ovalbumin and lysozyme (Oka and Schimke, 1969a,b), a function generally ascribed to estrogen (Kohler *et al.*, 1968, 1969; O'Malley *et al.*, 1969; McGuire and O'Malley, 1968). In both these cases (unstimulated and DES-withdrawn), there is no estrogen for progesterone to antagonize. Therefore, we see only the stimulatory events. When estrogen is present, however, the predominant response to progesterone is one of antagonism. Moreover, our results suggest that this antagonism takes place at the transcriptional level because progesterone suppresses production of estrogen-induced mRNAs and peptides.

Clearly progesterone exerts its influence on oviducts from unstimulated, DES-treated, or DES-withdrawn chicks in different manners. However, one salient feature of progesterone action occurs in all cases—the induction of avidin synthesis. Thus, induction of avidin synthesis emerges as a unifying concept regarding the mechanism of action of progesterone on the chick oviduct, and the studies presented in this investigation have contributed to elucidating the cytoplasmic events which occur subsequent to progesterone effects at the nuclear level but prior to the appearance of avidin molecules.

Although the mechanism by which progesterone induces the synthesis of avidin is still not entirely defined, our studies have progressed sufficiently to allow the following conclusions to be suggested: the primitive cells of the undifferentiated oviduct mucosa and later the differentiated goblet cells possess a specific protein receptor with a high affinity for progesterone (O'Malley *et al.*, 1970, 1971). Thus upon entering the cell progesterone is bound to a cytoplasmic receptor which subsequently moves into the nucleus (O'Malley *et al.*, 1970). Progesterone then causes alterations in nuclear rapidly labeled RNA synthesis (O'Malley *et al.*, 1969),

chromatin template capacity (O'Malley *et al.*, 1969, 1971), and RNA polymerase activity (McGuire and O'Malley, 1968). These alterations are paralleled by appearance of new species of hybridizable nuclear RNA (O'Malley and McGuire, 1968b,c, 1969). A conversion of ribosomes to polyribosomes then occurs, presumably due to the entry of new messenger RNA into the cytoplasm. These polyribosomes synthesize a qualitatively different population of peptides *in vitro* which presumably reflect earlier alterations in messenger RNA. Finally, the end product of this action of progesterone is the induction *de novo* of avidin synthesis. This sequence of events indicates, therefore, that progesterone exerts its primary effect on the nucleus to promote selective gene activation and subsequent synthesis of new avidin molecules. Studies designed to identify avidin molecules synthesized *in vitro* by oviduct polyribosomes are now in progress.

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Properties of a Polyriboadenylate Polymerase Isolated from Yeast Ribosomes*

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ABSTRACT: An enzyme isolated from yeast ribosomes catalyzes a primer-dependent synthesis of short polyriboadenylate chains from ATP. Other ribonucleoside triphosphates (UTP, GTP, and CTP) are not substrates, but when present individually or together with ATP result in inhibition of AMP polymerization. The reaction requires Mn^{2+} (10^{-3} M) or Mg^{2+} (10^{-2} M) for optimal activity. Yeast ribosomal RNA is a better primer than synthetic polyribonucleotides; yeast transfer RNA and calf thymus DNA (native or denatured) are inactive. Evidence is presented for the covalent linkage of the polyriboadenylate to the 3-hydroxyl end of the primer. The chain length of the polyriboadenylate (10–20 AMP residues) is dependent on time of incubation and primer RNA concentration.

Enzymes which catalyze the terminal polymerization of adenylate residues from ATP to the 3-hydroxyl end of polyribonucleotides have been detected in mammalian tissues (Edmonds and Abrams, 1960; Klemperer, 1963; Burdon, 1963), chick embryos (Venkataraman and Mahler, 1963), sea urchin embryos (Hyatt, 1967), and bacteria (August *et al.*, 1962; Gottesman *et al.*, 1962; Payne and Boezi, 1970). Although a physiological function for this type of polymerization reaction has not yet been defined, the isolation of adenylate-rich polynucleotides from rat liver (Hadjivassiliou and Brawerman, 1966), Ehrlich ascites cells (Edmonds and Cara-

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