# Changes in Chick Oviduct Ribonucleic Acid Following Hormonal Stimulation*

C. Wesley Dingman, Abraham Aronow, Sylvia L. Bunting, Andrew C. Peacock, and Bert W. O'Malley

ABSTRACT: Oviducts of immature chicks have been induced to differentiate by the *in vivo* administration of diethylstilbestrol or diethylstilbestrol plus progesterone. Nuclear and cytoplasmic ribonucleic acid preparations obtained from these oviducts were compared with similar preparations from oviducts of untreated chicks using polyacrylamide gel electrophoresis. No evidence was found in the cytoplasmic ribonucleic acid preparations for the production of new or unusual ribonucleic acid species concurrent with the diethylstilbestrol or diethylstilbestrol plus progesterone administration. Newly synthesized nuclear ribonucleic acid (labeled *in vitro*) from oviducts of diethylstilbestrol and diethylstilbestrol plus progesterone treated chicks showed a higher percentage of high molecular weight species than did nuclear ribonucleic acid from oviducts of untreated chicks. However, the most pronounced effect of the hormone treatment was a large increase in nuclear transfer ribonucleic acid and a definite but smaller increase in cytoplasmic transfer ribonucleic acid. This result was confirmed by assay of the amino acid acceptor activity of total nuclear and total cytoplasmic ribonucleic acid.

An early response of cells to steroid hormones has been an apparent increase in the rate of nuclear RNA synthesis (*e.g.*, Noteboom and Gorski, 1963; Ui and Mueller, 1963; Hamilton, 1964; Tata, 1966). When an attempt has been made to define which type or types of RNA are primarily affected, most of the evidence has suggested either that "ribosomal-type" RNA (Hamilton *et al.*, 1968) or that all types of RNA (Gorski and Nelson, 1965) are affected. There is little direct evidence for the induction of those specific species of mRNAs that act as the templates for the synthesis of the proteins induced in the target tissue by the administration of the hormone.

Estrogenic hormones have been shown to cause marked morphological changes in immature female chick oviducts (Hertz *et al.*, 1947) and to stimulate the differentiation of mature cell types (Kohler *et al.*, 1968a). The mucosa of the immature oviduct consists of a thin layer of pseudo-stratified columnar epithelium which rests upon a compact stroma of polygonal cells (Kohler *et al.*, 1968b). Within 24 hr after estrogen administration, marked stromal edema and ribosomal aggregation are evident. Ovalbumin secreting glands begin to develop at about day 4 of estrogen treatment as budlike invaginations of the original columnar epithelium. At day 6 of estrogen treatment, ovalbumin synthesis begins and by day 7, ciliated cells have also begun to appear in the surface epithelial layer. Goblet cells, which will synthesize avidin in response to progesterone administration, undergo proliferation after 9 days of estrogen treatment. Morphologic and biochemical differentiation is essentially complete by 18 days of estrogen administration.

The production by oviduct epithelium of various egg white proteins following estrogen administration has been previously reported (Brant and Nalbandov, 1956; O'Malley *et al.*, 1967). Progesterone administration, on the other hand, appears to stimulate only the synthesis of the specific chick oviduct protein, avidin (O'Malley, 1967; O'Malley *et al.*, 1967). In this report, we present data obtained by polyacrylamide gel electrophoresis of nuclear RNA from immature chick oviducts after stimulation with diethylstilbestrol or diethylstilbestrol plus progesterone. The most striking result was a large increase in nuclear tRNA, which was confirmed by analysis of acceptor activity. When newly synthesized RNA was analyzed following *in vitro* incubation with labeled precursors, there was also an increased amount of heterogeneous, high molecular weight, nuclear RNA, particularly in those oviducts from chicks treated with both diethylstilbestrol and progesterone.

## Experimental Section

*Materials.* Pancreatic ribonuclease A (salt free, lyophilized) and pancreatic deoxyribonuclease I (electrophoretically purified, RNase free) were the products of Worthington Biochemical Corp. Sarkosyl NL-97 (sodium lauroyl sarcosinate) was a gift of the Geigy Chemical Corp. [$^3$H]5-Uridine (20 Ci/mmole) and [$^3$H]cytidine (15 Ci/mmole) were purchased from Schwarz BioResearch, Inc. [$^3$H-Methyl]methionine (100 mCi/mmole), [$^{14}$C]amino acid mixture, and [$^3$H]cytidine (5.4 Ci/mmole) were purchased from New England Nuclear Corp. Tissue culture medium 199 was obtained from Microbiological Associates, diethylstilbestrol from Merck and Co., and progesterone from Eli Lilly and Co.

*Preparation of Oviduct Tissues and Incubation with Labeled Precursors.* Female Rhode Island Red chicks (3-day old) were injected subcutaneously with 5 mg of diethylstilbestrol in sesame oil daily for 17 days (preparation E) and killed on the day following the last injection. An equal number of chicks were treated similarly with diethylstilbestrol but also received an additional injection of progesterone (5 mg subcutaneously) 14 hr prior to sacrifice (preparation E + P). Immature

* From the Chemistry Branch and the Endocrinology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014. *Received August 28, 1968.*

(no hormone treatment, preparation U) oviducts were removed from chicks of the same age (*i.e.*, 21 days). The animals were killed by subluxation of the cervical vertebrae. The oviducts were removed and either used immediately for the preparation of RNA or stored at −70°. *In vitro* labeling of oviduct RNA was accomplished by incubation of lightly minced tissue in medium 199. All groups (U, E, and E + P) were incubated in the presence of 25 μCi/ml of [$^3$H]uridine and 12 μCi/ml of [$^3$H]-cytidine under conditions of 95% $O_2$–5% $CO_2$ for 25 or 50 min at 38°. Incubations were terminated by freezing the tissue at −70°.

*Preparation of Embryonic and Adult Chicken Tissues.* Embryonic chick tissues were obtained from fresh, fertile, New Hampshire Red eggs (Duckworth Hatcheries, Hanover, Md.) which had been incubated to the desired age. Embryonic chick brains (17-day old) were labeled *in vivo* by intravenous injection of 100 μl of [$^3$H]cytidine in aqueous solution (1 μCi/μl). Adult, New Hampshire Red laying hens were obtained from the same source as the fertile eggs. White Leghorn pullets (122- and 140-days old) were obtained from Amstutz Hatchery, Westminster, Md.

*Isolation of Nuclear and Cytoplasmic RNA.* Cytoplasmic RNA was isolated and its concentration determined as described previously (Peacock and Dingman, 1967). Nuclei were isolated in most cases as described by Lazarus and Sporn (1967) for mouse liver except that the first crude nuclear pellet was washed twice with solution 1 (0.32 M sucrose–0.001 M potassium phosphate–0.002 M $MgCl_2$, pH 6.8), washed three times with solution 2 (0.32 M sucrose–0.001 M potassium phosphate–0.001 M $MgCl_2$, 0.3% Triton N101, pH 6.3), and finally washed once with solution 1. The nuclear preparations were free of gross cytoplasmic contamination as judged by phase microscopy. In some cases, following two washes with solution 1, the nuclei were purified by isopycnic sedimentation in solution 3 (2.1 M sucrose containing 0.002 M $MgCl_2$, and 0.001 M potassium phosphate, pH 6.8; Sporn *et al.*, 1962), and in other cases nuclei were purified by the modified Lazarus and Sporn technique as described above, followed by sedimentation through solution 3. Nuclear RNA was isolated as described previously (Dingman and Peacock, 1968).

*Electrophoretic Analysis of RNA on Acrylamide and Mixed Acrylamide–Agarose Gels.* The electrophoretic techniques, including the preparation of RNA samples and their treatment with enzymes, the preparation of acrylamide and mixed acrylamide–agarose gels, the staining procedure, and the sectioning and counting of gel strips containing labeled RNA samples, were as described previously (Dingman and Peacock, 1968; Peacock and Dingman, 1968).

*Assay of Amino Acid Acceptor Activity.* The amino acid acceptor activity of RNA samples was determined as follows. Aliquots of RNA (approximately 150 μg in 0.75 ml of solution 4 (0.05 M NaCl–0.001 M NaEDTA, pH 6.2)) were first incubated at 37° for 15 min with 0.1 ml of a solution containing 200 μg of DNase/ml and which was 0.02 M NaCl–0.008 M $MgCl_2$–0.001 M potassium phosphate (pH 7.0). Then 0.33 ml of a solution was added which was 0.066 M Tris, 0.032 M $MgCl_2$, 0.032 M dithiothreitol, 0.0012 M $Na_2ATP$, and 0.022 M $K_2HPO_4$ (pH 7.6), and which contained 15 μCi of [$^{14}$C]amino acids and 50 μCi of [$^3$H]methionine. Freshly prepared 220,000*g* rat liver supernatant fraction was added (0.1 ml) and the entire mixture was incubated for 15 min at

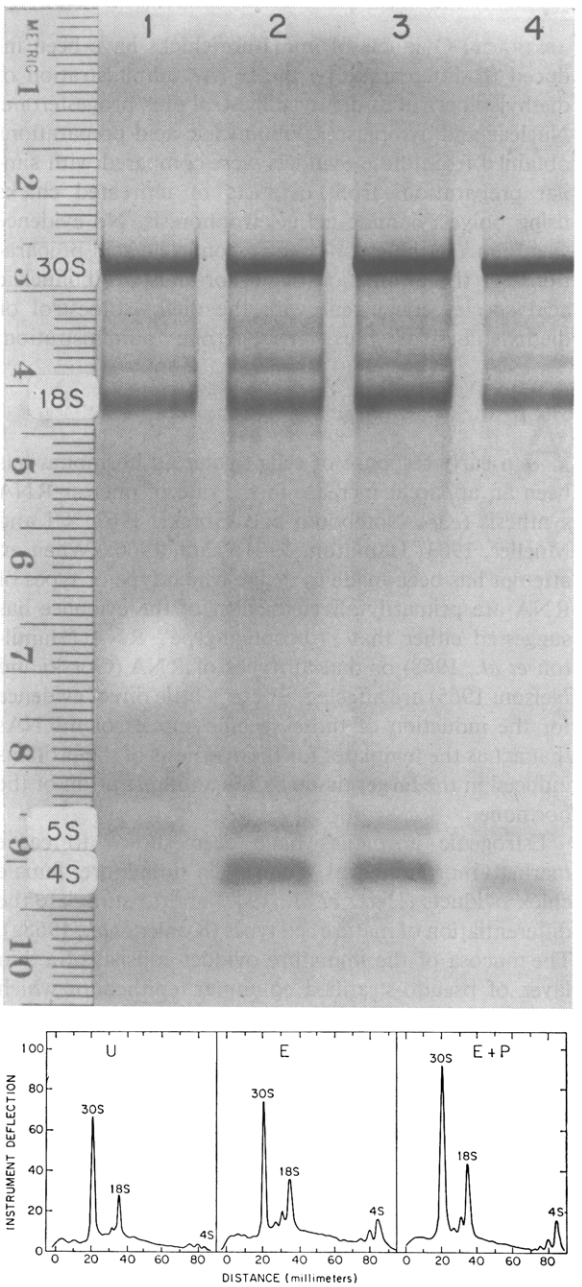


FIGURE 1: Electrophoregrams (in a 2.0% polyacrylamide plus 0.5% agarose gel run for 1.75 hr) of chick oviduct nuclear RNAs, DNase treated. (A) Photograph of stained gel. From left to right, the samples applied were: slot 1, nuclear RNA from preparation U; slot 2, nuclear RNA from preparation E; slot 3, nuclear RNA from preparation E + P; slot 4, nuclear RNA from preparation E + P (same preparation as slot 3, except 0.67 as much applied). The position of the 30S, 18S, 5S, and 4S RNA is indicated on the photograph. (B) Densitometric scans (using a Photovolt densitometer) of the stained gel shown in (A), slots 1–3; the origin in these scans was arbitrarily set at the point corresponding to 7 mm in the photograph.

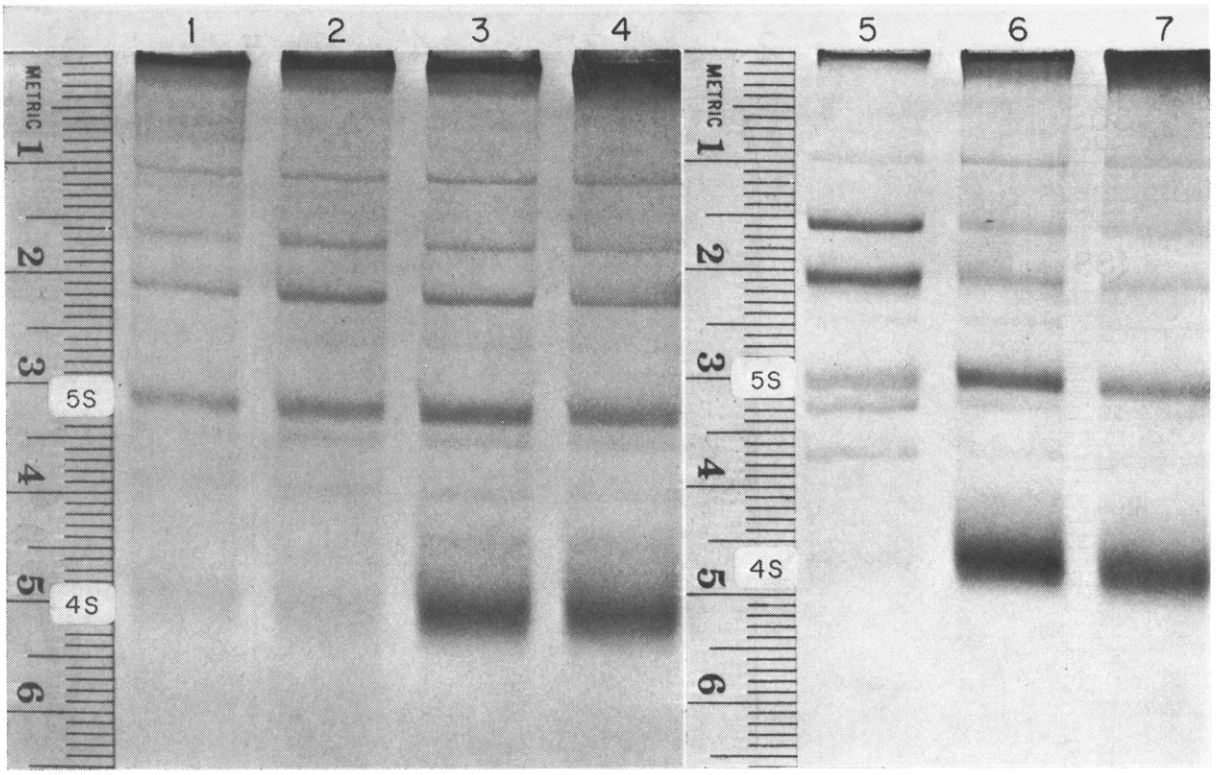


FIGURE 2: Electrophoregrams (in a 10% polyacrylamide gel run for 3.5 hr) of chick oviduct and chick brain nuclear RNAs. From left to right, the samples applied were: slot 1, nuclear RNA from preparation U; slot 2, nuclear RNA from a different preparation U; slot 3, nuclear RNA from preparation E; slot 4, nuclear RNA from preparation E + P; slot 5, nuclear RNA from 8-day embryonic chick brain; slot 6, nuclear RNA from preparation E; slot 7, nuclear RNA from preparation E + P.

37°. The reaction was stopped by the addition of 1.0 ml of 1% Sarkosyl NL-97 and chilling to 0°. Samples of oviduct RNA (150 $\mu$g) were added at this point to those control reaction mixtures which had no oviduct RNA present during the incubation. The RNA was then extracted with 3.0 ml of water-saturated phenol and the resulting aqueous phase was dialyzed exhaustively against solution 4. The specific activity of each sample was then determined.

Additional controls on the validity of the assay system were performed. The use of a boiled supernatant enzyme preparation, brief treatment of the reaction mixture with alkali (a treatment insufficient to lower the recovery of RNA), omission of ATP, or preincubation of the RNA with RNase for 15 min at 37° resulted in very low specific activities relative to that obtained from the usual assay system (*i.e.*, 0.03, 0.05, 0.10, and 0.15, respectively, for RNA samples from oviduct preparation E). These results confirmed the specificity of the reaction for the assay of amino acid acceptor activity in the tested RNA samples.

The rat liver supernatant fraction was prepared as described previously (Dingman and Peacock, 1968) except that the final centrifugation was performed at 220,000*g* (average) for 1 hr.

## Results

*Analytical Studies.* In order to compare the composition of RNA preparations from the different tissues, approximately equal amounts of RNA were applied to the gel. The most striking finding in these studies was the relatively larger amounts of tRNA in the nuclei of those oviduct preparations (E and E + P) from chicks that had been treated with diethylstilbestrol than in the nuclei from oviducts of untreated chicks. This is illustrated in Figures 1 and 2. This increase was observed whether the nuclei were purified by the Lazarus and Sporn (1967) technique, isopycnic sedimentation, or a combination of these methods. There was also a definite but smaller increase in the relative amount of tRNA present in the cytoplasm of oviduct preparations from the diethylstilbestrol-treated chicks (E and E + P) and this is illustrated in Figure 3. The increased tRNA, as observed on the stained electrophoregrams of both nuclear RNA and cytoplasmic RNA, was present whether the tissue had been processed fresh or after storage at −70°. All of the stained material in the tRNA region of the electrophoregrams was sensitive to RNase treatment and insensitive to DNase treatment.

High percentages of tRNA were found in nuclear RNA from oviducts of immature chicks treated with diethylstilbestrol or diethylstilbestrol plus progesterone, and in normally maturing oviducts from 140-day-old pullets. In contrast, low percentages of tRNA were found in nuclear RNA from brain and liver of the embryonic chick, from liver of immature chicks treated with diethylstilbestrol, from oviducts of 122-day-old pullets, and from liver and oviducts of adult laying hens.

Comparison of various nuclear RNA preparations

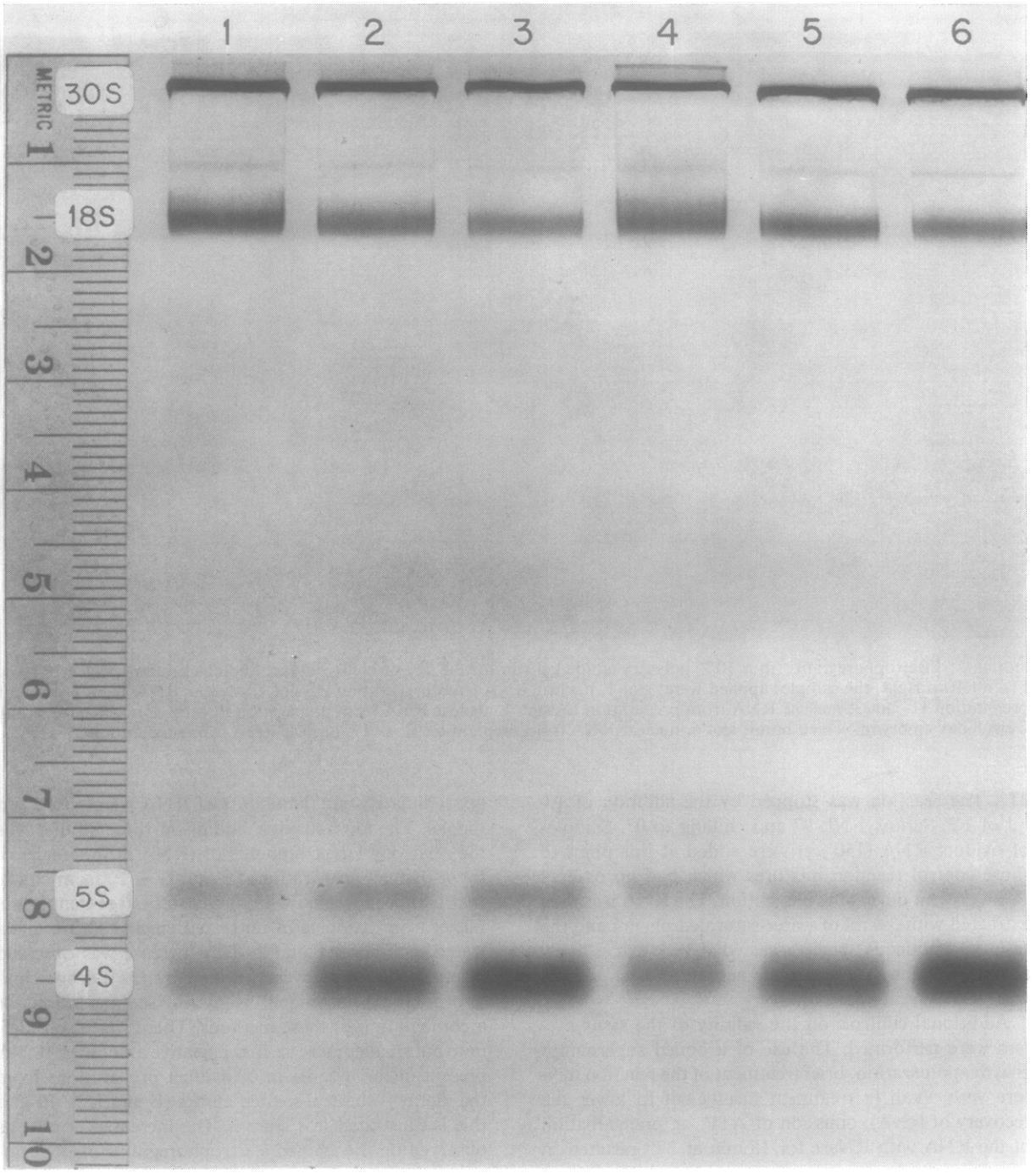


FIGURE 3: Electrophoregrams (in a 3.0% polyacrylamide plus 0.5% agarose gel run for 2 hr) of chick oviduct cytoplasmic RNAs. From left to right; the samples applied were: slot 1, cytoplasmic RNA from preparation U; slot 2, cytoplasmic RNA from preparation E; slot 3, cytoplasmic RNA from preparation E + P; slot 4, cytoplasmic RNA from preparation U (a different preparation than run in slot 1); slot 5, cytoplasmic RNA from preparation E (a different preparation than run in slot 2); slot 6, cytoplasmic RNA from preparation E + P (a different preparation than run in slot 3).

by polyacrylamide gel electrophoresis has indicated that there are striking differences between animal species in the band pattern obtained from the low molecular weight classes (4–10 S) of nuclear RNA (*cf.* Figure 2 this paper with Figure 2 of Dingman and Peacock, 1968). However, the patterns given by the low molecular weight species of nuclear RNA are apparently the same in preparations from different organs of the same animal, although such RNA preparations may differ with respect to the amount of RNA present in a particular band.

*Amino Acid Acceptor Activity of RNA Preparations.* To confirm that the increased staining in the region occupied by tRNA on the electrophoregrams of the E and E + P preparations was a reflection of an increased con-

TABLE I: Amino Acid Acceptor Activity of Nuclear and Cytoplasmic RNAs from Immature Female Chick Oviducts.

| RNA | Prior Treatment of Chicks (see text) | Yield[a] ($\mu$g) | $^3$H (cpm/$\mu$g) | $^{14}$C (cpm/$\mu$g) | $\Delta^3$H, Sp Act.[b] | $\Delta^{14}$C, Sp Act.[b] |
|---|---|---|---|---|---|---|
| Nuclear | U | 147 | 9 | 128 | 5 | 14 |
| Nuclear | E | 160 | 13 | 191 | 9 | 77 |
| Nuclear | E + P | 139 | 15 | 225 | 11 | 111 |
| Cytoplasmic | U | 161 | 14 | 173 | 9 | 53 |
| Cytoplasmic | E | 164 | 21 | 270 | 16 | 150 |
| Cytoplasmic | E + P | 172 | 26 | 340 | 21 | 220 |
| Nuclear control[c] | E + P | 162 | 4 | 114 | | |
| Cytoplasmic control[c] | E | 154 | 5 | 120 | | |

[a] These figures represent the total amount of RNA recovered after phenol extraction and dialysis of the incubation mixture. [b] These figures represent the difference between the experimental groups and the control incubations (see footnote *c* to this table) and therefore are a measure of the total amount of tRNA present in each RNA preparation. [c] These two incubations served as controls in which the RNA was added after the reaction was stopped (see Experimental Section).

tent of tRNA, the amino acid acceptor activity of a number of the RNA preparations was examined. To control for the presence of tRNA in the rat liver supernatant fraction used as a source of amino acid acylating enzymes, two incubations were performed in which the oviduct RNA was omitted during the incubation and then added after the reaction had been stopped. These results are shown in Table I. The amino acid acceptor activity, measured either with [$^3$H]methionine or [$^{14}$C]-amino acids, in RNA preparations from both nucleus and cytoplasm, was highest in oviducts from those chicks treated with both diethylstilbestrol and progesterone. It was somewhat lower in oviducts from chicks treated with diethylstilbestrol alone, and was lowest in oviducts from untreated chicks. The amino acid acceptor activity in nuclear RNA preparations from this latter group was similar to that found previously in nuclear RNA from adult rat liver (Dingman and Peacock, 1968). These results thus confirm the impression gained from the electrophoregrams (Figures 1–3).

*In Vitro Labeling Studies.* Short-term (25 and 50 min) *in vitro* labeling of oviduct RNA was carried out with the hope that if new species of RNA were being produced in oviducts from animals treated with E and E + P, these new RNAs might be detected by gel electrophoresis.

After 50-min incubation with label, the electrophoretic distribution of labeled cytoplasmic RNA from all three oviduct preparations was the same: the 30S, 18S, and 4S RNAs were clearly labeled, and no other significant bands were seen. The ratio of 30S $^3$H counts to 18S $^3$H counts was approximately 1.3 in all cases. These results resembled those found earlier for rat liver cytoplasmic RNA after 60 min of *in vivo* labeling (Dingman and Peacock, 1968).

In the case of the newly synthesized nuclear RNAs, the electrophoretic patterns obtained from the three oviduct preparations differed significantly. These differences are illustrated in Figure 4 in which the nuclear

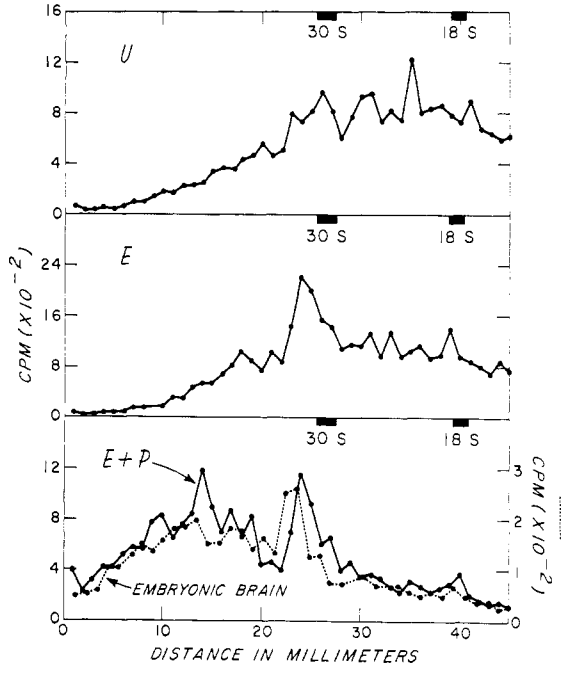


FIGURE 4: Labeling patterns obtained by electrophoresis for 1.75 hr (in 2% polyacrylamide plus 0.5% agarose gels) of chick oviduct and chick brain nuclear RNAs. Preparations U, E, and E + P are defined in the Experimental Section. Each has been labeled *in vitro* with [$^3$H]cytidine and [$^3$H]uridine for 50 min. The embryonic brain preparation is from 17-day embryonic chick brain labeled *in vivo* with [$^3$H]cytidine for 30 min.

RNAs have been examined using 2.0% acrylamide plus 0.5% agarose gels. After 50-min *in vitro* incubation with labeled RNA precursors, the amount of label found as high molecular weight RNA (migrating less rapidly than 30 S) was greatest in E + P, less in E, and quite low in U. The percentage of the total counts found in the 45 mm of the gel shown in Figure 4, that is in RNA equal

to or larger than 18S RNA, was 76, 53, and 35%, respectively. When the nuclear RNAs from these same oviducts were electrophoresed in 10% acrylamide gels (not shown) in which only the lower molecular weight species are resolved, the recovery of counts, down to and including 4S RNA, was 28, 17, and 6% for nuclear RNA preparations from U, E, and E + P, respectively. After 25 min of label, all three oviduct RNA preparations showed a higher percentage of label in the higher molecular weight range, consistent with earlier reports (Penman, 1966; Dingman and Peacock, 1968), but showed the same relative distribution with respect to treatment. To provide a comparison, 17-day embryonic chick brain nuclear RNA was labeled *in vivo* with [$^3$H]cytidine for 30 min. The pattern of labeling (Figure 4) resembled most closely that of nuclear RNA from oviducts of chicks treated with E + P after 50 min of *in vitro* labeling. Therefore, the pattern of newly synthesized nuclear RNA found in oviducts from untreated immature chicks (preparation U) is not necessarily typical of chick nuclear RNA.

Of possible significance is the observation that none of the *in vitro* labeling studies revealed any increase, in either the nucleus or the cytoplasm, in the amount of *newly synthesized* tRNA in RNA preparations from E and E + P chicks. It is possible that low molecular weight degradation products of newly synthesized, high molecular weight, nuclear RNA obscured small changes in the relative amount of newly synthesized tRNA in nuclear RNA from preparations E and E + P, or that the labeling time was too short to reveal a relative increase in its rate of synthesis. Alternatively, the rate of accumulation of tRNA may have diminished by the 18th day of estrogen treatment (O'Malley *et al.*, 1968).

## Discussion

The patterns of newly synthesized nuclear RNAs found after 25 and 50 min of *in vitro* labeling cannot be interpreted clearly. Although the predominance of larger RNA molecules in E + P clearly reflects the effects of hormone treatment, it is also clear that E + P is the more "usual" result (see also Hiatt, 1962; Penman, 1966), and that the rather low percentage of counts found in the high molecular weight RNA of oviducts from preparation U is somewhat unusual. It is possible that the results in preparations E and E + P represent synthesis of mRNAs. It is also possible (since some of these larger RNA molecules are known to be ribosomal precursors) that the E + P treatment is more effective in stimulating formation of ribosomes than either the E or U treatments (Hamilton *et al.*, 1968). Finally, it may be that diethylstilbestrol and progesterone directly or indirectly protect newly synthesized nuclear RNA from degradation (particularly during *in vitro* incubation). In any case, the more stable RNAs probably remain intact, because visual examination of the stained electrophoregrams of the nuclear RNA preparations from *in vitro* incubated oviducts did not reveal signs of significant degradation.

Our inability to detect new or unusual species of newly synthesized RNA in the cytoplasm of hormonally stimulated oviducts may be a result of (a) insufficient resolving power of acrylamide gel electrophoresis; (b) covalent attachment of new mRNA species to one of the species of rRNA (Gould *et al.*, 1966; Hadjiolov, 1967); or (c) the use of a labeling period too short to permit the completion and/or transport to the cytoplasm of new RNA molecules (Hadjiolov, 1967).

The observation that the nuclear RNA from normally maturing oviducts of 140-day-old pullets had an increased amount of tRNA suggests that the changes in tRNA induced in immature chick oviducts by exogenously administered hormones is similar to the normal process. The return to normal amounts of tRNA in nuclear RNA preparations from oviducts of adult laying hens suggests that the increase in tRNA is related more to the differentiation than to the function of the adult organ. It may be that the estrogen and progesterone effects on tRNA are restricted to oviduct cells. Increased amounts of nuclear tRNA were not found in the livers of chicks treated with E and E + P (although this organ is affected by estrogen treatment; Greengard *et al.*, 1965).

We do not know whether the relative increase in tRNA observed after hormonal stimulation is a result of increased synthesis, decreased degradation, or a combination of these processes. Nor do we know if there has been a change in the distribution of specific tRNAs present or merely a proportional increase in each of the species of tRNA present in the unstimulated oviducts. In this connection, there have been a number of reports of changes in the distribution of specific tRNAs in tissues undergoing differentiation (*e.g.*, Kaneko and Doi, 1966; Holland *et al.*, 1967; Lee and Ingram, 1967; Yang and Comb, 1968; Vold and Sypherd, 1968). There is also a report (Bendaña and Galston, 1965) of an apparent hormone-induced stabilization of tRNA (*i.e.*, a reduced rate of degradation) in pea stem tissues and it is possible that a similar effect is being observed in our oviducts treated with E and E + P.

With respect to the control of protein synthesis and the induction of specific proteins by estrogen and progesterone, it would be helpful to know whether or not tRNA was in some way rate limiting for *in vivo* protein synthesis in these tissues. In the immature chick oviduct stimulated with diethylstilbestrol or diethylstilbestrol plus progesterone and in the normally maturing oviduct of the pullet, it may be the availability of specific tRNAs which regulates message translation at the cytoplasmic level.

## References

Bendaña, F. E., and Galston, A. W. (1965), *Science 150*, 69.

Brant, J. W. A., and Nalbandov, A. V. (1956), *Poultry Sci. 35*, 692.

Dingman, C. W., and Peacock, A. C. (1968), *Biochemistry 7*, 659.

Gorski, J., and Nelson, N. J. (1965), *Arch. Biochem. Biophys. 110*, 284.

Gould, H. J., Arnstein, H. R. V., and Cox, R. A. (1966), *J. Mol. Biol. 15*, 600.

Greengard, O., Sentenac, A., and Acs, G. (1965), *J. Biol. Chem. 240*, 1687.
Hadjiolov, A. A. (1967), *Progr. Nucleic Acid Res. Mol. Biol. 7*, 195.
Hamilton, T. H. (1964), *Proc. Natl. Acad. Sci. U. S. 51*, 83.
Hamilton, T. H., Widnell, C. C., and Tata, J. R. (1968), *J. Biol. Chem. 243*, 408.
Hertz, R., Larsen, C. D., and Tullner, W. (1947), *J. Natl. Cancer Inst. 8*, 123.
Hiatt, H. H. (1962), *J. Mol. Biol. 5*, 217.
Holland, J. J., Taylor, M. W., and Buck, C. A. (1967), *Proc. Natl. Acad. Sci. U. S. 58*, 2437.
Kaneko, I., and Doi, R. H. (1966), *Proc. Natl. Acad. Sci. U. S. 55*, 564.
Kohler, P. O., Grimley, P. M., and O'Malley, B. W. (1968a), *Science 160*, 86.
Kohler, P. O., Grimley, P. M., and O'Malley, B. W. (1968b), *J. Cell Biol.* (in press).
Lazarus, H. M., and Sporn, M. B. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 1386.
Lee, J. C., and Ingram, V. M. (1967), *Science 158*, 1330.
Noteboom, W. D., and Gorski, J. (1963), *Proc. Natl. Acad. Sci. U. S. 50*, 250.
O'Malley, B. W. (1967), *Biochemistry 6*, 2546.
O'Malley, B. W., Aronow, A., Peacock, A. C., and Dingman, E. W. (1968), *Science 162*, 567.
O'Malley, B. W., McGuire, W. L., and Korenman, S. G. (1967), *Biochim. Biophys. Acta 145*, 204.
Peacock, A. C., and Dingman, C. W. (1967), *Biochemistry 6*, 1818.
Peacock, A. C., and Dingman, C. W. (1968), *Biochemistry 7*, 668.
Penman, S. (1966), *J. Mol. Biol. 17*, 117.
Sporn, M. B., Wanko, T., and Dingman, W. (1962), *J. Cell Biol. 15*, 109.
Tata, J. R. (1966), *Progr. Nucleic Acid Res. Mol. Biol. 5*, 191.
Ui, H., and Mueller, G. C. (1963), *Proc. Natl. Acad. Sci. U. S. 50*, 256.
Vold, B. S., and Sypherd, P. S. (1968), *Proc. Natl. Acad. Sci. U. S. 59*, 453.
Yang, S. S., and Comb, D. G. (1968), *J. Mol. Biol. 31*, 139.

# Stokes Radius of Human Pituitary Hormones and Demonstration of Dissociation of Luteinizing Hormone*

Robert J. Ryan

ABSTRACT: A technical modification, namely the use of radioiodine-labeled proteins, has been used in the gel filtration technique for the determination of the Stokes radius of human pituitary follicle-stimulating hormone, luteinizing hormone, somatotrophic hormone, and thyroid-stimulating hormone. Data are presented to justify the use of this technical modification. The Stokes radii of these pituitary hormones were found to be 32.2 Å for follicle-stimulating hormone, 22.2 Å for somatotrophic hormone, and 27.5 Å for thyroid-stimulating hormone.

Two species of luteinizing hormone molecules were found, the larger having a Stokes radius of 30.2 Å and the smaller, 22.6 Å. The molecular weights of these species of luteinizing hormone were estimated to be 28,000 and 14,400.

Porath and Flodin (1959) described the technique of separating materials of different molecular sizes by passing a solution of the substances through a bed of porous gel with solvent phases inside and outside of the gel grains. Whitaker (1963) and Andrews (1964) proposed that this technique could be used for estimating the molecular weight of unknown proteins by comparing their elution volumes with those of proteins of known molecular weight. However, it became apparent (Laurent and Killander, 1964; Siegel and Monty, 1966; Squire, 1964) that this estimation of molecular weight was valid only when the unknown protein had a partial specific volume and a frictional ratio similar to those of the standards used. Evidence was provided that the elution position of a protein during gel filtration was related to the Stokes radius of the molecule (an index of its size) rather than to its molecular weight.

The present study was undertaken to determine the Stokes radius of human pituitary luteinizing hormone, follicle-stimulating hormone, thyroid-stimulating hormone, and somatotrophic hormone. These measurements were performed with radioiodinated preparations of the hormones and the validity of this technical modification was established. In addition evidence is presented for the existence of associated and dissociated forms of human luteinizing hormone.

* From the Mayo Clinic and Mayo Foundation: Section of Endocrine Research, Rochester, Minnesota 55901. *Received July 15, 1968.* This investigation was supported in part by Research Grant HD-3726 from the National Institutes of Health, U. S. Public Health Service, and a grant from the Mayo Foundation.