HORMONAL INDUCTION OF SPECIFIC PROTEINS IN CHICK OVIDUCT CELL CULTURES

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The mechanisms of hormone action and regulation are complex and difficult to examine in the whole animal or perfused organ. Cell cultures have less intercellular orientation and are composed of a more homogeneous cell population. It would be desirable to study induction of protein synthesis directly in these relatively simple systems. Only a few such systems exist; among these are induction of new enzyme protein in hepatoma suspension culture (Thompson, et. al., 1966) or induction of enzyme activity in cell monolayer culture (Griffin and Cox, 1966). The present study demonstrates the effect of steroid hormones on induction of specific proteins in monolayer cell cultures of chick oviduct tissue. Diethylstilbestrol stimulated ovalbumin production and introduction of progesterone into the cell culture induced synthesis of a specific protein, avidin. The incubation product was avidin by biological, immunological, and electrophoretic (disc-gel) criteria.

MATERIALS & METHODS

Four day old female Rhode Island Red chicks were injected subcutaneously with 5 mg diethylstilbestrol daily for 18 days. The chicks were then sacrificed and 1 mm diameter explants of epithelial tissue from the mucosal

surface of the oviducts were placed in 30 ml plastic flasks[1] with a surface area of 25 cm^2. Minced oviduct was also treated with 0.25% trypsin in Dulbecco's tris medium without calcium or magnesium and started as dispersed cell cultures. The cells were allowed to grow in Parker's 199 medium with 20% fetal bovine serum and 50 units of Pencillin and 50 µg Streptomycin mixture (Microbiological Laboratories) per ml for a period of 6 weeks prior to testing. At that time, a mixed monolayer culture of epithelioid cells and fibroblasts had completely covered the surface of the flasks and complete changes of media were made every 1 or 2 days throughout the study. After a control period, 5 µg of diethylstilbestrol per ml was added to the media for 14 days, followed by a 12 day period of incubation with 5 µg of progesterone per ml of medium.

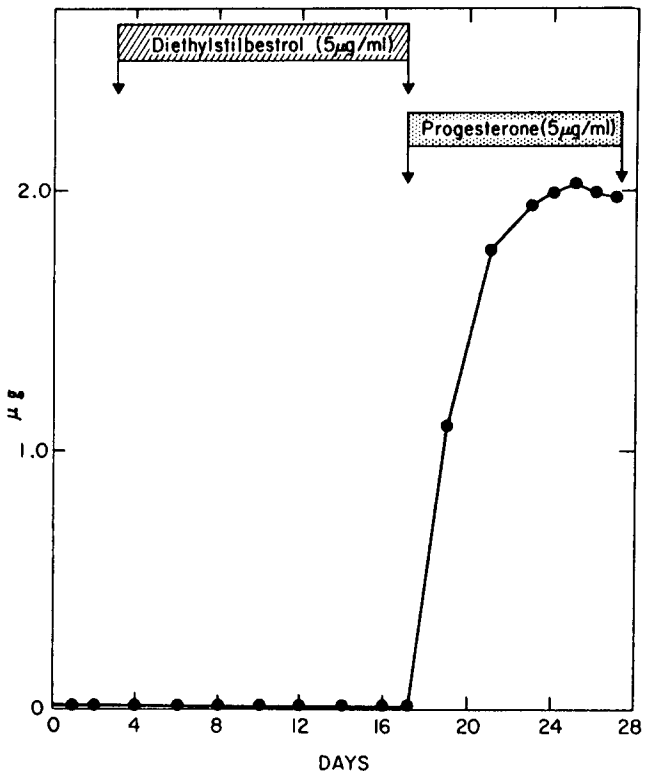
Avidin was quantified by a recently described assay (Korenman & O'Malley, 1967) involving specific binding of the avidin to D-biotin-carboxyl-^{14}C, adsorption of the avidin-biotin-^{14}C complex onto bentonite, and trapping the complex on a millipore filter.

Ovalbumin was precipitated by specific antibody and quantified by protein estimation of the precipitate using the Folin-Lowry procedure (Kabat and Mayer, 1961). Agar gel diffusion studies on Ouchterlony plates demonstrated that the large bulk of precipitating protein was identical with ovalbumin. Lysozyme was assayed by the method of Litwack (1955).

RESULTS & DISCUSSION

The results of a sample experiment demonstrating hormonal induction of avidin in chick oviduct monolayer cultures are shown in Fig. 1.

[1]Falcon Plastics, B-D Laboratories, Inc., Baltimore, Maryland



No measurable avidin was present in the incubation medium during 3 control days or during 14 days of continuous diethylstilbestrol stimulation. On the first medium change following cessation of diethylstilbestrol and introduction of progesterone into the incubating medium, avidin induction occurred. The maximum stimulation occurred during the first 48 hours and approached a plateau after the sixth day.

To demonstrate that the biotin-binding protein was also immunologically identical to avidin, studies were carried out utilizing a double-antibody immunoassay (O'Malley and Korenman, unpublished data). New Zealand rabbits were injected with commercially purified avidin (Worthington) and avidin antiserum was obtained. Aliquots of the medium were incubated with the avidin antiserum and ^{14}C-labeled biotin. It has been previously shown that

biotin does not interfere with an avidin-antiserum reaction, nor is the binding capacity of avidin for biotin altered by a prior attachment of antibody to the avidin molecule (Siva Sankar, et. al., 1958). Sheep anti-rabbit globulin was then added and the resulting precipitate was washed, dissolved in NCS solubilizer (Nuclear Chicago), and counted in a toluene-phosphor solution. The results are shown in Table 1. No immunological identifiable avidin was noted in control or diethylstilbestrol monolayer culture media. Avidin induction again occurred when progesterone was added to the cell culture. The dose response curve was linear with respect to both increasing amounts of cell media and added avidin standard.

Disc gel electrophoresis was employed as a third criterion for avidin identity. Gels were run at pH 4.5 (7% gel) where the bulk of the culture media protein remained in the upper third of the gel but the avidin-biotin-^{14}C complex ran in the bottom third. Gels were sliced into equal 1.3 mm slices and hydrolyzed with hydrogen peroxide or NCS solubilizer and counted (Fig. 2).

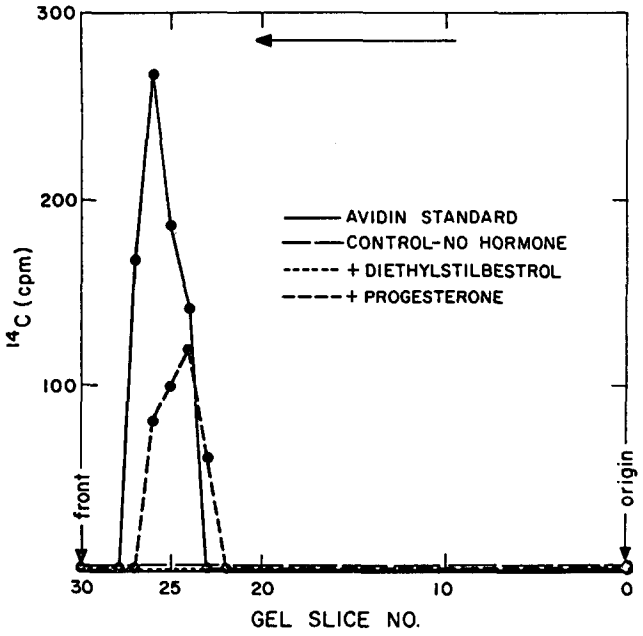


TABLE 1

IMMUNOLOGICAL IDENTITY OF AVIDIN

Cell Culture Medium	Amount of Media (ml)	^{14}C biotin bound* to antibody pp't (cpm)	Avidin per sample (µg)
I			
Control - No Hormone	0.1	0	-
" " "	0.4	0	-
II			
Diethylstilbestrol (5µg/ml)	0.1	0	-
" "	0.4	0	-
III			
Progesterone (5µg/ml)	0.1	33	0.1
" "	0.2	70	0.22
" "	0.4	126	0.39
IV			
III without Avidin Antiserum	0.1	0	-
" " " "	0.4	0	-
Avidin Standard Added to I (0µg)	-	0	-
" " " " " (0.1µg)	-	34	-
" " " " " (0.5µg)	-	159	-
" " " " " (1.0µg)	-	336	-

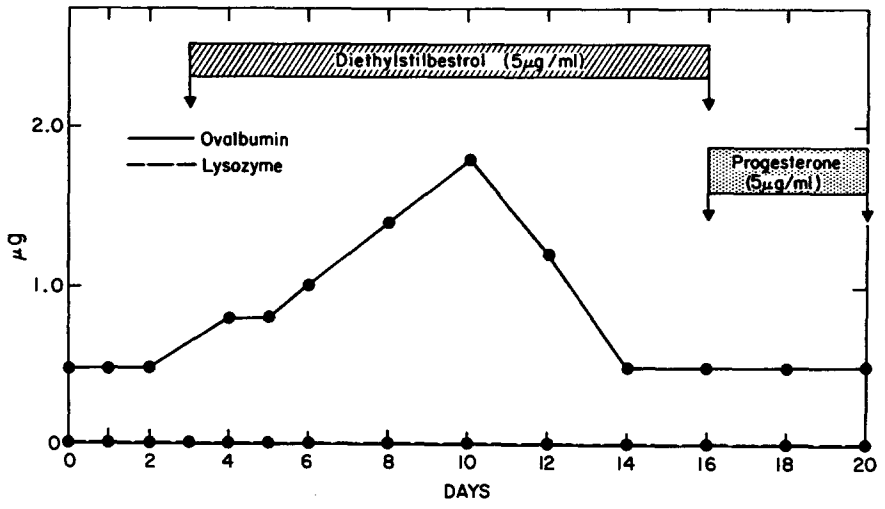
* Aliquots of monolayer cell culture media were reacted with 100 µl of rabbit avidin antiserum for 2 hours at room temperature. An excess of D-carboxyl-^{14}C-biotin (1250 cpm) was added and the reaction continued for 20 min. Then ml of sheep anti-rabbit globulin was added and the mixture was allowed to stand for 3 hours. The ppt. was spun and washed x4 with buffer (0.01M phosphate, 0.9% NaCl, pH 7.2) and dissolved and counted.

The progesterone-treated cell cultures secreted a material electrophoretically identical with an avidin standard, while no detectable avidin-like material was present in control or diethylstilbestrol-treated cultures.

The possibility that previously synthesized avidin was only released and not synthesized de novo during progesterone administration was ruled out by analyses of subcellular fractions showing no intracellular accum-

mulation of avidin until progesterone was introduced. Experiments involving induction of avidin synthesis have been repeated on over 40 different monolayer cultures. Approximately 80% of the total cultures prepared have functioned to produce avidin. Avidin synthesis did not occur in cultures of oviduct sub-epithelial stroma.

The monolayer cell cultures were also examined for ovalbumin induction under the influence of diethylstilbestrol. A typical experiment is shown in Fig. 3.



There was a small amount of baseline ovalbumin synthesis seen during the control period followed by an increase in the immunologically precipitable ovalbumin after introduction of diethylstilbestrol into the cell culture. A peak was reached on the tenth day followed by a gradual decline to baseline even though exposure to diethylstilbestrol was continued. The mechanism of this decline is not yet understood. No change in baseline synthesis occurred during progesterone treatment.

Lysozyme activity was not detectable in the cell incubation media at any time during the experiment (Fig. 3). Lysozyme is a normal intracellul

constituent of oviduct tissue. A rise in activity would be expected if cell damage occurred.

In summary, we have demonstrated: (1) that after 3 months in monolayer culture, chick oviduct tissue was capable of differentiative function; (2) that diethylstilbestrol stimulated ovalbumin production into the incubation medium; (3) that introduction of progesterone into the cell culture induced synthesis of avidin; (4) that this new protein was biologically, immunologically, and electrophoretically identical to authentic avidin standard. This system promises to be a simple, efficient and reliable tool for the study of hormonal control of protein synthesis.

REFERENCES

Griffin, M. J., and Cox, R. P., Proc. Natl. Acad. Sci., 56, 946, (1966).

Kabat, E. A., and Mayer, M. M., in Experimental Immunochemistry, Thomas, Springfield, Illinois, (1961), p. 361.

Korenman, S. G., and O'Malley, B. W., Biochim et Biophys. Acta (in press).

Litwack, G., Proc. Soc. Exp. Biol. Med., 89, 401, (1955).

Siva Sankar, D. V., Cossano, H. W., Theis, H. W., and Marks, C. R., Nature, 181, 619, (1958).

Thompson, E. B., Tomkins, G. M., and Curran, J. F., Proc. Natl. Acad. Sci., 56, 296, (1966).