Decreased ovalbumin-gene response to oestrogen in the prenatally diethylstilboestrol-exposed chick oviduct

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Prenatal exposure of the female chick-embryo Müllerian duct to diethylstilboestrol (DES) decreases its future capacity for epithelial tubular-gland-cell differentiation and oviduct ovalbumin-gene expression. Chick Müllerian ducts after prenatal exposure to different concentrations of DES were tested after birth to determine the response of the oviduct toward the oestrogen induction. The quantity of DNA was biochemically determined, the differentiation of tubular-gland cells in the oviduct was studied by light microscopy, and the expression of the ovalbumin gene was detected by hybridization of the total RNA with radioactive ovalbumin cDNA. Comparisons among these three parameters revealed that the expression of the ovalbumin gene was affected most by DES exposure. Exposure to high doses of DES suppressed ovalbumin-gene expression by 75–78%, and inhibited tubular-gland-cell differentiation and thus decreased the DNA content by 29 and 32% respectively. Exposure to low doses of DES caused suppression of ovalbumin-gene expression by 47–53%, but it did not affect the other two parameters. Prenatal DES exposure has strong inhibitory effects on the Müllerian duct at the age (5–8-day-old embryos) when the organ is undifferentiated. Less inhibition is observed when the organ becomes differentiated (15-day-old embryos and older).

Oestrogens are essential for the normal differentiation and development of the female reproductive system in the vertebrate embryo. However, excessive amounts of these compounds have adverse effects. The association of prenatal DES (a synthetic stilbene oestrogen) exposure with genital-tract abnormalities in female offspring is one of the best-known examples of such effects. It has been confirmed in avian and mammalian species that excess amounts of DES in the embryo can cause permanent damage to the genitals. In the chick, prenatal DES exposure causes an alteration in the pattern of sexual differentiation and a decrease in the oestrogenic responsiveness system (Teng & Teng, 1979). In rodent species, abnormalities, e.g. adenosis-like lesions, adenocarcinoma, stromal-cell sarcoma and leiomyosarcoma, were

found in several strains of mice exposed to DES prenatally (Plapinger & Bern, 1979; McLachlan *et al.*, 1980). Treatment of pregnant hamsters with DES on days 14 and 15 of gestation led to hyperplastic and neoplastic lesions in the sex tracts of most female progeny (Rustia & Shubik, 1979). In primates, cervical hooding and vaginal adenosis were discovered in the genital tracts of most of the pubertal female rhesus monkeys exposed prenatally to DES (Hendrickx *et al.*, 1978). In humans, several cases of vaginal adenocarcinomas were found in patients aged 15–23 years, and further investigations showed that almost all of them had been exposed to DES during their foetal life (Herbst & Scully, 1970; Greenwald *et al.*, 1971; Herbst *et al.*, 1972, 1979; Kaufman *et al.*, 1980; Orenberg, 1981). Unfortunately, the cause of and mechanism for the prenatal toxicity of DES remain unresolved.

To gain more insight into this problem, it is necessary to know the changes in some major biochemical events and the developmental of hormone-responsiveness systems in the oviduct at the embryonic stage. In the past decade, we and

Abbreviations used: DES, diethylstilboestrol; SDS, sodium dodecyl sulphate; mRNA_{ov}, ovalbumin mRNA.

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others have established the female chick oviduct as the model system for this type of study. This model has the following advantages: (1) the ontogeny of the receptor and acceptor systems in the Müllerian duct (the embryonic precursor for the future chick oviduct) is well studied (Teng & Teng, 1975*a,b*, 1978*a,b*; Teng, 1980); (2) in the Müllerian duct, the normal pattern of differentiation at the genomic and morphological levels is well documented (Andrews & Teng, 1979*a,b*); (3) the hormonal secretion in embryonic gonads is well understood (Weniger & Zeis, 1971; Teng & Teng, 1977; Guichard *et al.*, 1977); and (4) the postnatal effect of steroid hormones on changes in various biochemical parameters in chick oviduct is well established (Palmiter, 1972, 1973; Means & O'Malley, 1974). Above all, the epithelial tubular-gland cell is the major cell type in the oviduct after oestrogen stimulation, and the differentiation as well as the expression of the ovalbumin gene in the tubular-gland cell are modulated by oestrogen.

This study provides the answers to the question of whether prenatal DES exposure could affect the future target organ's gene expression. The experiments were conducted to elucidate whether (1) the concentration of DES exposure *in ovo* plays an important role in determining the ovalbumin gene's response to oestrogen stimulation, and whether (2) in a specific stage of development the genital tract is vulnerable to the effects of DES. Part of this work was presented at the 12th Annual Meeting of the American Society for Cell Biology (Teng & Teng, 1982).

Materials and methods

Chemicals

The following chemicals, hormones and enzymes were obtained from the sources indicated: [³H]dGTP (17 Ci/mmol) was purchased from Schwarz/Mann (Orangeburg, NY, U.S.A.); [³H]dCTP (24 Ci/mmol) and [³H]dATP (13 Ci/mmol) were from New England Nuclear (Boston, MA, U.S.A.); poly(rU) carrier was from P-L Biochemicals (Milwaukee, WI, U.S.A.); oligo-(dT)-cellulose was from Collaborative Research Inc. (Lexington, MA, U.S.A.); DES was from

Eastman Kodak Co. (Rochester, NY, U.S.A.); actinomycin D, oestradiol-17 β and S₁ nuclease were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); avian-myeloblastosis-virus reverse transcriptase was given by Dr. J. W. Beard of Life Sciences (St. Petersburg, FL, U.S.A.). All other chemicals were of analytical grade.

Animals

Fertilized White Leghorn chick eggs were incubated and maintained as described previously (Teng & Teng, 1978*a,b*). The hatched female chicks were kept in battery cages, fed on the normal balanced ration diet and given water *ad libitum*.

The chick embryos were initially exposed to DES *in ovo* at various concentrations at day 5. Embryos were treated by a dipping method (Teng & Teng, 1979). In this method, the bottom half of the egg is partially immersed in DES dissolved in 70% (v/v) ethanol maintained at 50–55°C for 10s. The concentration of DES ranged from 2.5 to 10 mg/ml of solution. The treated eggs were returned to the incubator until hatching. The details of methodology, optimal dosage and length of time for DES exposure have been described by Andrews & Teng (1979*a*). At 1 week after hatching, the young chicks were treated with a second dose of oestradiol-17 β (500 μ g/chick) for 5 or 15 days. The embryonic DES exposure and the postnatal oestradiol treatment are summarized in Scheme 1. The magnum region of the oviducts from the control and hormone-treated chicks was removed and immediately frozen in liquid N₂. The frozen tissue was broken into small pieces and homogenized in 5 vol. of SDS buffer (0.5% SDS/25 mM-EDTA/75 mM-NaCl, pH 8.0). The total RNA in the homogenate was isolated as described by Andrews & Teng (1979*a*).

Isolation of purified ovalbumin mRNA

The mRNA_{ov} was purified from the total nucleic acid extract of hen oviducts as described by Woo *et al.* (1975), with some modifications. First, an oligo(dT)-cellulose column chromatography was used to adsorb the poly(A)-containing mRNA. Next, to prevent aggregation between mRNA and

Scheme 1. Time schedule for embryonic DES exposure and postnatal oestradiol treatment

rRNA, the RNA was heated at 70°C in 1.0 mM- $\text{Na}_2\text{-EDTA}$, pH 7.0, for 1 min, followed by rapid cooling to 4°C before chromatography on the Sepharose 4B column and the second oligo(dT)-cellulose column (Rosen *et al.*, 1975). This technique replaced the nitrocellulose filtration method. The final purification of mRNA_{ov} was by chromatography on a 2% agarose column containing 6M-urea/0.025M-sodium citrate buffer, pH 3.5, in a Buchler Poly-Prep electrophoresis apparatus. Some 3–6 mg of purified mRNA_{ov} was generally obtained from 500 g of tissue in each preparation. The purity of the ovalbumin mRNA was assessed by acid/urea/agarose-gel electrophoresis and sucrose-gradient centrifugation in 70% formamide. Throughout the purification procedure, mRNA_{ov} and total mRNA activities were determined by the cell-free wheat-germ translation assay. By the mRNA_{ov} determination procedure of Woo *et al.* (1975), the mRNA_{ov} is approx. 90% pure.

Preparation of cDNA

The synthesis of ^3H -labelled DNA complementary to ovalbumin mRNA (cDNA_{ov}) was modified from the procedure of Harris *et al.* (1975). The reaction mixture (final vol. 0.5 ml) contained 50 mM-Tris/HCl (pH 8.1), 10 mM-MgCl₂, 36 μg of actinomycin D/ml, 20 mM-dithiothreitol, 150 μM - ^3H dATP, 83 μM - ^3H dCTP, 120 μM - ^3H dGTP, 0.5 mM-dTTP, 2.5 μg of (dT)_{12–18}, 50 μg of mRNA_{ov} and 4 μg of sodium pyrophosphate/ml. The components were mixed and preincubated for 5 min at 0°C, and 150 units of avian-myeloblastosis-virus-RNA-dependent DNA polymerase were added (Kacian & Myers, 1976). Incubation was for 60 min at 41°C. The reaction was terminated by adding 0.5% SDS, 10 mM-EDTA and 1% (w/v) of tRNA. The ^3H cDNA_{ov} was separated from the uncharged deoxynucleotides by passing the reaction mixture through a Sephadex G-50 column (80 cm $\times$ 1.5 cm) equilibrated with 0.1 M-NaCl/2 mM-EDTA. The peak fractions were pooled and precipitated with 2 vol. of ethanol. The precipitate was then dissolved in 0.2 M-NaOH/10 mM-EDTA and heated at 75°C for 15 min to destroy mRNA. It was then rapidly cooled to room temperature. The solution was neutralized with 1 M-Tris, pH 7.5, and the ^3H cDNA_{ov} with carrier yeast rRNA (50 μg /ml) were precipitated with 2 vol. of ethanol at –20°C for 18 h. The final precipitate was dissolved in distilled water to a concentration in which 2.0 ml represented about 3000 c.p.m. or about 45 pg of ^3H cDNA_{ov}.

Hybridization in the presence of RNA excess

The hybridization reactions in the presence of RNA excess was as described by Harris *et al.*

(1975) and Andrews & Teng (1979b). The reaction mixture consists of 0.6 M-NaCl, 10 mM-Hepes, pH 7.6, 2 mM-EDTA, 1.0 ng of single-stranded ^3H cDNA_{ov}/ml (6×10^7 c.p.m./ μg ; a portion of the cDNA used for the initial part of the experiments was given by Dr. M. J. Tsai, Cell Biology Department, Baylor College of Medicine, Houston, TX, U.S.A.), with total RNA samples ranging from 0.1 to 2000 μg /ml depending on the sample being analysed, and 400 μg of poly(rU) carrier/ml. The reaction mixture was adjusted to a final volume of 50 μl , sealed in a conical glass vial, heated for 2 min at 100°C, then incubated at 68°C for 0.5–96 h. Samples were taken at different time points and analysed for total trichloroacetic acid-precipitable radioactivity and S₁-nuclease-resistant radioactivity in the presence and absence of the RNA sample. mRNA_{ov} could protect 86–90% of the total radioactivity from S₁-nuclease digestion; 10–12% of the total trichloroacetic acid-precipitable radioactivity was resistant to S₁ nuclease at zero time and in the absence of RNA.

S₁-nuclease treatment of hybrids

At the end of hybridization, 450 μl of S₁ buffer [100 mM-NaCl/100 mM-sodium acetate buffer (pH 4.5)/1 mM-ZnCl₂/denatured calf thymus DNA (50 μg /ml)] and 5 units (1 unit solubilizes 1 μg of DNA/min) of S₁ nuclease were added to the reaction mixture. The reaction was allowed to proceed at 37°C for 1 h. It was then stopped by the addition of 100 μg of bovine serum albumin, followed by 3 ml of cold 20% (w/v) trichloroacetic acid. After 30 min at 4°C, the contents in the vial were collected and washed on Millipore filters. The filters were dried and dissolved in 3 ml of methyl-Cellosolve. The radioactivity in the filters was assayed in the presence of 10 ml of Aquasol (New England Nuclear) with a Beckman model LS-250 scintillation spectrometer at 35% efficiency.

The data obtained from these hybridization experiments in the presence of excess RNA are expressed as the percentage of ^3H cDNA_{ov} that was resistant to S₁-nuclease digestion (% hybrid) versus the product of the RNA sample concentration (mol/l) $\times$ time (s), or R_{0t} . The graphed data have been corrected for the non-hybridizing components of the ^3H cDNA_{ov} probe.

General biochemical procedures

Protein was determined by the procedure described by Lowry *et al.* (1951), with bovine serum albumin (Sigma) as a standard. DNA was determined by the diphenylamine reaction (Giles & Myers, 1965), with calf thymus DNA (Schwarz/Mann) as a standard. RNA was determined by the procedure reported by Munro & Fleck (1966).

Purified RNA samples were measured by the A_{260} ; 1 mg of RNA/ml has A_{260} 25.

Histological preparations

Chick oviducts were removed, washed in 0.9% NaCl and fixed in Bouin's fixative. Tissue samples were rinsed in 70% (v/v) ethanol before being embedded in paraffin wax. Histological sections (5 μ m) from the tissue were stained with Haematoxylin and Eosin. Three to five different chick oviducts per group were assayed for the percentage of tubular-gland cells in the magnum by microscopic examination of both longitudinal and cross-sectional slides through the magnum region. At least 500 cells were counted in each determination.

Results and discussion

Initially, the embryos were treated with DES at 2.5–5 mg/ml of dipping solution. The content of nucleic acids and the hybridization experiments in the presence of RNA excess were performed with total cell RNA extracts in order to examine the oestrogenic response of the oviduct to prenatal DES exposure (Fig. 1).

After 5 days of postnatal oestradiol stimulation, the content of total RNA per duct remained the same for the control and the prenatally DES-exposed duct. However, the percentage of mRNA_{ov} in the total RNA population was

decreased by approx. 30% in the prenatally DES-exposed duct (Table 1). The average number of mRNA_{ov} molecules per tubular-gland cell was 10800–12300 in DES-exposed oviducts, compared with 23000 molecules in the non-exposed oviducts. This is comparable with what has been found in newborn-chick oviducts after 4 days of DES stimulation; 20000 molecules of mRNA_{ov}/cell was induced in the magnum region (Harris *et al.*, 1975). The results indicated that, after exposure to these doses of DES, the synthesis of mRNA_{ov} in the tubular-gland cell of the oviduct was decreased by 47–53%. However, a slight increase in DNA content and in the number of differentiated tubular-gland cells were observed (Table 1). The dose of DES used for the treatment is considered a relatively lower dose in our experimental system. In our previous experiment, we found that dipping eggs in this concentration of DES solution for a definite time period allowed a constant amount of DES (approx. 0.00013%) intake into the serum of the embryo (Teng & Teng, 1979; Andrews & Teng, 1979a). Our previous finding indicated that prenatal treatment with a low dose of DES acts as a differentiating agent in chick Müllerian duct (Andrews & Teng, 1979b).

Further investigation showed that prenatal exposure at a higher dose of DES (10 mg/ml in the dipping solution) to the day-5 embryo suppresses the oviduct for future response to oestradiol

Table 1. *Effects of prenatal DES treatment on the development of the chick oviduct magnum region*
Embryos were exposed to DES on day 5 (DES₁, 2.5 mg/ml solution; DES₂, 5 mg/ml solution) and stimulated with oestrogen (oestradiol, E₂) after birth. The details were described in the legend to Fig. 1 and in the Materials and methods section. RNA and DNA contents were determined for the magnum region as described in the Materials and methods section. Values are means $\pm$ S.D. for five determinations. Five different females per group were assayed for the percentage of tubular-gland cells in the magnum by microscopic examination of both longitudinal and cross-sectional slides through the magnum region. At least 500 cells were counted in each determination. Values represent the means for three to five determinations. The number of mRNA_{ov} molecules per tubular-gland cell was estimated by using the following equations:

$$a = \text{no. of mRNA}_{ov} \text{ molecules/duct} = \text{RNA content/duct} \times \% \text{ of mRNA}_{ov} \times \frac{\text{Avogadro's number}}{M_r \text{ of mRNA}_{ov}}$$

$$b = \text{no. of tubular-gland cells/duct} = \frac{\text{magnum DNA content}}{\text{DNA content/cell}^*} \times \% \text{ of tubular-gland cells/magnum}$$

$$a/b = \text{no. of mRNA}_{ov} \text{ molecules/tubular-gland cell}$$

Sample	RNA/duct (μ g)	DNA/duct (μ g)	Percentage of mRNA _{ov} †	Percentage of tubular-gland cells‡	Molecules of mRNA _{ov} § per tubular-gland cell	
					(no./cell)	(% decrease)
Oviduct (no DES + E ₂)	3314 $\pm$ 48	587 $\pm$ 49	0.070	43 $\pm$ 5	23 000 $\pm$ 3800	0
Oviduct (DES ₁ + E ₂)	3280 $\pm$ 47	632 $\pm$ 50	0.055	58 $\pm$ 6	12 300 $\pm$ 2600	47
Oviduct (DES ₂ + E ₂)	3142 $\pm$ 48	646 $\pm$ 53	0.051	61 $\pm$ 6	10 800 $\pm$ 2000	53

* 2.5 μ g/cell (Sober, 1968).

† Values were determined by RNA excess hybridization in Fig. 1.

‡ The identification of tubular-gland cells is described by Kohler *et al.* (1969).

§ 6.3×10^5 g/mol (Woo *et al.*, 1975).

Fig. 1. Determination of mRNA in the prenatally DES-exposed chick oviduct which received 5 days of oestrogen stimulation

Embryonic DES treatment was at day 5. Two groups of embryos were individually treated with 2.5 or 5 mg of DES/ml. The control group was treated with dipping solution without DES. After hatching, the chicks from these three groups were individually stimulated with oestrogen for 5 days as detailed in Scheme 1. The procedures for RNA-excess hybridization and the measurement of the percentage of hybrid were described in the Materials and methods section. Equivalent R_0t values are corrected for the effects of salt concentration on the reaction rate (Britten & Smith, 1969). The R_0t curves represent hybridization reactions between [^3H]cDNA and RNA of the following origins: ∇ , mRNA_{ov}; $\bullet$, total RNA from animals without prenatal DES exposure; $\blacktriangle$, total RNA from animals treated with 2.5 mg of DES/ml and with 5 days of oestradiol stimulation; $\blacksquare$, total RNA from animals treated with 5 mg of DES/ml and with 5 days of oestradiol stimulation; $\circ$, total RNA from chick oviducts without prenatal DES and postnatal oestrogen treatment; $\triangle$, total RNA from chick oviducts treated prenatally with 2.5 mg of DES/ml but without postnatal oestrogen stimulation.

Fig. 2. Determination of mRNA in the prenatally DES-exposed chick oviduct which received 15 days of oestrogen stimulation

Embryos were treated with DES at different stages of development by the dipping method. All eggs were treated with 10 mg of DES/ml, whereas the control group received the same treatment without DES. At 1 week after hatching, the chicks were stimulated with 500 μg of oestradiol-17 β daily for 15 days. The extraction of total RNA from the oviducts and the hybridization of RNA to cDNA_{ov} were detailed in the Materials and methods section. The R_0t curves represent hybridization reactions between [^3H]cDNA and the total RNA from the following sources: $\blacksquare$, animals without prenatal DES exposure but with 15 days of oestradiol stimulation; ∇ , animals with prenatal DES exposure at day 15; $\blacktriangle$, animals with prenatal DES exposure at day 12; $\bullet$, animals with prenatal DES exposure at day 8; $\triangle$, animals with prenatal DES exposure at day 5. At 1 week after hatching, all of these DES-pretreated animals have received 15 days of oestradiol stimulation. $\square$, Total RNA from the chick oviducts without prenatal and postnatal DES (or oestrogen) treatment; $\circ$, total RNA from animals treated with 10 mg of DES/ml at day 12 without postnatal oestrogen stimulation.

stimulation (Fig. 2 and Table 2). After 15 days of oestradiol stimulation, there were 18611 mRNA_{ov} molecules/cell in the oviduct that had been exposed to DES at day 5 of embryonic age, compared with 85000 mRNA_{ov} molecules/cell in the duct with no DES exposure. This was a drastic decrease (78%) in the ovalbumin-gene expression by prenatal DES exposure. In addition, both DNA and RNA contents were decreased, by 32% and 52% respectively, compared with that without DES pretreatment (Table 2). This was in contrast with previous observations that prenatal exposure to a lower concentration of DES produced no inhibitory effect but a slight stimulation of DNA content (Table 1). Studies of radioimmunoassay indicated that an egg dipped in this solution (10 mg/ml) had received 5 μg of DES/ml in the serum of the embryo; the DES concentration in the embryo was

5 times that absorbed by exposure to a lower dose of DES (Teng & Teng, 1979). This and previous experiments indicated that in the chick oviduct there probably exists a threshold of response to prenatal DES obstruction. A higher dose of DES is required to decrease the content of nucleic acids and the differentiation of epithelial tubular-gland cells, whereas a lower dose of DES causes only a decrease in ovalbumin-gene expression.

Chick embryos at days 5, 8, 12 and 15 of incubation were exposed to DES, and their response to oestrogen stimulation was investigated postnatally. The numbers of mRNA_{ov} molecules per cell induced by oestrogen were 19000, 21600, 41500 and 60000 respectively, compared with 85000 molecules induced in the oviduct that received no prenatal DES exposure (Table 2): significant decreases of 78, 75, 51 and 30%, respectively, were observed. The oestrogenic ef-

Table 2. Age-dependent effects of prenatal diethylstilboestrol on the development of the chick oviduct magnum region. The embryonic DES exposure and oestrogen stimulation after birth were detailed in the legend to Fig. 2. Determination of RNA, DNA, percentage of tubular-gland cells and number of mRNA_{ov} molecules per tubular-gland cell were described in the legend of Table 1. 'Newborn control' means newborn control female chick with no prenatal DES treatment. R_{0t} represents the rate of reaction between [³H]cDNA and ovalbumin mRNA. 'Fraction of mRNA' is the percentage of mRNA_{ov} in total cell RNA from oestrogen-treated or control sex tract. The data were calculated by comparing the initial slope of the titration curve for sample RNA with that observed with pure mRNA_{ov} (Young *et al.*, 1974).

Sample	R_{0t}	Fraction of mRNA	RNA		DNA		Proportion of tubular-gland cells		Molecules of mRNA _{ov} per tubular gland cell	
			(μ g/duct)	(% decrease)	(μ g/duct)	(% decrease)	(%)	(% decrease)	(no./cell)	(% decrease)
Newborn control	0.95	3.2×10^{-3}	4560	0	528	0	80	0	85000	0
DES at 15 days	1.40	2.1×10^{-3}	4000	12	512	3	70	13	59900	30
DES at 12 days	2.20	1.4×10^{-3}	3520	23	484	8	64	20	41520	51
DES at 8 days	3.60	8.3×10^{-4}	2160	53	360	32	58	28	21550	75
DES at 5 days	4.40	6.8×10^{-4}	2200	52	358	32	57	29	18611	78

fects on other parameters, i.e. RNA and DNA contents, and the numbers of differentiated tubular-gland cells, were also decreased (by 52, 53, 23 and 12%; 32, 32, 8 and 3%; 29, 28, 20 and 13%, respectively) (Table 2). Among these four developmental stages, it is evident that the oestrogen effect on ovalbumin-gene expression and the content of nucleic acids was greatly decreased by prenatal DES exposure at days 5 and 8 of embryonic age. However, a lesser effect was observed in day-12 and -15 embryos.

In parallel to this observation, the inhibition of [³H]thymidine incorporation into Müllerian vagina epithelial DNA caused by neonatal oestrogen exposure has been demonstrated in the mouse (Eide, 1975; Iguchi *et al.*, 1976). Morphologically, prenatal DES exposure caused developmental arrest and structural alteration in the mouse uterus and oviduct (McLachlan *et al.*, 1980; Newbold *et al.*, 1983). The results reported in the present paper are the first indication that genomic expression in the sex tract is also subject to DES inhibition.

At 5 and 8 days of chick embryos, the Müllerian duct is not differentiated, yet after day 15 of embryonic age, the Müllerian duct is fully differentiated, as indicated by its response to the hormone-mediated genomic activation for transcription and translation (Teng & Teng, 1978a,b). All of the existing information indicates that the stability created in the Müllerian-duct genome after day 15 of embryonic age explains why it is more resistant to the exogenous DES effect.

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References

- Andrews, G. K. & Teng, C. S. (1979a) *Biochem. J.* **182**, 257-269
- Andrews, G. K. & Teng, C. S. (1979b) *Biochem. J.* **182**, 271-286
- Britten, R. J. & Smith, J. (1969) *Year Book Carnegie Inst. Washington* **68**, 378-395
- Eide, A. (1975) *Cell Tissue Res.* **156**, 551-555
- Giles, K. W. & Myers, A. (1965) *Nature (London)* **206**, 93
- Greenwald, P., Barlow, J. J., Nasca, P. C. & Burnett, W. S. (1971) *N. Engl. J. Med.* **285**, 390-392
- Guichard, A. L., Cedard, L., Mignot, T. M., Scheib, D. & Haffen, K. (1977) *Gen. Comp. Endocrinol.* **32**, 255-265
- Harris, S. E., Rosen, J. M., Means, A. R. & O'Malley, B. W. (1975) *Biochemistry* **14**, 2072-2081
- Hendrickx, A. G., Benirschke, K., Thompson, R. S., Ahern, J., Lucas, W. E. & Oi, R. (1978) *Teratology* **17**, 23A
- Herbst, A. L. & Scully, R. E. (1970) *Cancer* **25**, 745-757
- Herbst, A. L., Kurman, R. J. & Scully, R. E. (1972) *Obstet. Gynecol. (N.Y.)* **40**, 287-298
- Herbst, A. L., Scully, R. E. & Robboy, S. (1979) *Natl. Cancer Inst. Monogr.* **51**, 25-35
- Iguchi, T., Ohta, Y. & Takasugi, N. (1976) *Dev. Growth Differ.* **18**, 69-78
- Kacian, D. L. & Myers, J. C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2191-2195
- Kaufman, R. H., Adam, E., Binder, G. L. & Gerthoffer, E. (1980) *Am. J. Obstet. Gynecol.* **137**, 299-308
- Kohler, P. O., Grimley, P. M. & O'Malley, B. W. (1969) *J. Cell Biol.* **40**, 8-27
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275
- McLachlan, J. A., Newbold, R. R. & Bullock, B. C. (1980) *Cancer Res.* **40**, 3988-3999
- Means, A. R. & O'Malley, (1974) *Biochem. Cell Differ.: MTP Int. Rev. Sci.* **9**, 161-180
- Monahan, J. J., Harris, S. E. & O'Malley, B. W. (1976) *J. Biol. Chem.* **251**, 3738-3748

- Munro, H. N. & Fleck, A. (1966) *Methods Biochem. Anal.* **14**, 113–176
- Newbold, R. R., Tyrey, S., Haney, A. F. & McLachlan, J. A. (1983) *Teratology* **27**, 417–426
- Orenberg, C. L. (1981) *DES: The Complete Story*, pp. 34–53, St. Martin's Press, New York
- Palmiter, R. D. (1972) *J. Biol. Chem.* **247**, 6450–6461
- Palmiter, R. D. (1973) *J. Biol. Chem.* **248**, 2095–2106
- Plapinger, L. & Bern, H. A. (1979) *J. Natl. Cancer Inst.* **63**, 507–518
- Rosen, J. M., Woo, S. L. C., Holder, J. W., Means, A. R. & O'Malley, B. W. (1975) *Biochemistry* **14**, 69–78
- Rustia, M. & Shubik, P. (1979) *Cancer Res.* **39**, 4636–4644
- Sober, H. A. (ed.) (1968) *Handbook of Biochemistry*, p. H-57, Chemical Rubber Co., Cleveland
- Teng, C. S. (1980) in *The Developmental of Responsiveness to Steroid Hormones* (Kaye, A. M. & Kaye, M., eds.), vol. 25, pp. 77–94, Pergamon Press, Oxford
- Teng, C. S. & Teng, C. T. (1975a) *Biochem. J.* **150**, 183–190
- Teng, C. S. & Teng, C. T. (1975b) *Biochem. J.* **150**, 191–194
- Teng, C. S. & Teng, C. T. (1978a) *Biochem. J.* **172**, 361–370
- Teng, C. S. & Teng, C. T. (1978b) *Biochem. J.* **176**, 143–149
- Teng, C. S. & Teng, C. T. (1979) in *The Ontogeny of Receptor and Reproductive Hormone Action* (Hamilton, T. H., Clark, J. H. & Sadler, D., eds.), pp. 421–440, Raven Press, New York
- Teng, C. S. & Teng, C. T. (1982) *J. Cell Biol.* **95**, 183a
- Teng, C. T. & Teng, C. S. (1977) *Biochem. J.* **162**, 123–134
- Weniger, J. P. & Zeis, A. (1971) *Gen. Comp. Endocrinol.* **16**, 391–397
- Woo, S. L., Rosen, J. M., Liarakos, C. D., Choi, Y. C., Busch, H., Means, A. R., O'Malley, B. W. & Robberson, D. L. (1975) *J. Biol. Chem.* **250**, 7027–7039
- Young, B. D., Harrison, P. R., Gilmour, R. S., Birnie, G. D., Hell, A., Humphries, S. & Paul, J. (1974) *J. Mol. Biol.* **84**, 555–568