Studies on Sex-Organ Development

ISOLATION AND CHARACTERIZATION OF AN OESTROGEN RECEPTOR FROM CHICK MÜLLERIAN DUCT

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An oestradiol-binding macromolecule was observed in the left Müllerian duct of the 15-day female chick embryo. The embryonic receptor binds oestradiol with a high affinity and low capacity, having a K_d of $3.2 \times 10^{-9} \text{ M}$ and a maximal number of sites of $5.45 \text{ fmol}/10^6$ cells in the left Müllerian duct. The receptor is protein in nature, as suggested by its susceptibility to proteolysis; in addition, it is organ- and steroid-specific. Judging by glycerol-gradient analysis, the hormone receptors in the cytosol are present in 8S and 4.5S forms, and the 8S form could be dissociated into a 4.5S form in the presence of 0.5 M-KCl . A 4.5–6S receptor could be extracted from the nuclei. Under physiological salt conditions, the embryonic receptors bind to DNA-cellulose and can be eluted when the salt concentration is increased to 0.5 M-KCl . Determination by isoelectric focusing indicates that the isoelectric point is 5.8 for the 8S and 6.9 for the 4.5S receptor.

The properties of steroid-hormone receptors (Giannopoulos & Gorski, 1971*a,b*; Fang *et al.*, 1969; Gardner & Tomkins, 1969; Schrader & O'Malley, 1972) and their mechanism of action in the target organs (Mainwaring & Peterken, 1971; Steggle *et al.*, 1971; King & Gordon, 1972; Higgins *et al.*, 1973) have been carefully studied in recent years. This knowledge has given the developmental biologist a deeper insight into the molecular events involved in hormonal control of normal organ differentiation. Both the male and female 5–6-day chick embryos possess undifferentiated pairs of Müllerian ducts. In the male embryo, the right and left Müllerian ducts start involution on the 8th day of incubation and disappear by the 13th day. In the female embryo, the right Müllerian duct starts to regress on the 9th day of incubation and by the time of hatching, appears as a tiny cloacal stump. The left Müllerian duct, however, continues to develop, eventually becoming the functional oviduct of the mature female chick. The secretion of steroid hormones by the gonads and their effect on the course of differentiation of the reproductive system in the chick embryo have been thoroughly investigated (Gallien & LeFoulogoc, 1957; Weniger, 1958; Haffen & Cedard, 1968). The involvement of steroid hormones in Müllerian duct development has long been an interesting topic for study (Wolff & Wolff, 1951; Hamilton, 1963; Raynaud, 1970), yet the important questions about the interaction of the sex steroids and this embryonic target organ have never been asked. The present study demonstrated and documented the presence, isolation and properties of an oestradiol receptor in the Müllerian duct of the embryonic female chick. A preliminary abstract

of some of these findings has been presented elsewhere (Teng & Teng, 1974).

Materials and Methods

Animals and chemicals

Fertile white-Leghorn-chicken eggs, obtained from Rich-Glo Farms, Houston, Tex., U.S.A., were placed in a humidified incubator (Petersime model 4) at 38°C . Left Müllerian ducts excised from 15-day embryos (Hamburger & Hamilton, 1951) were used in all experiments. $[6,7\text{-}^3\text{H}]$ Oestradiol-17 β (specific radioactivity 45 Ci/mmol ; New England Nuclear Corp., Boston, Mass., U.S.A.), supplied in solution in benzene-methanol (9:1, v/v), was evaporated to dryness and redissolved in ethanol before use. Solutions of unlabelled oestradiol, diethylstilboestrol, aldosterone, testosterone, progesterone, oestriol, oestrone and cortisol (Sigma Chemical Co., St. Louis, Mo., U.S.A.) were made up in ethanol, and dilutions were made with buffer (containing 10 mM-Tris-HCl and 1.5 mM-EDTA , pH 7.4). The following chemicals were purchased from the sources indicated: bovine serum albumin and proteinase (*Streptomyces griseus*) (Sigma), 7S bovine γ -globulin, dextran and ribonuclease (bovine pancreas) (Schwarz/Mann, Orangeburg, N.Y., U.S.A.), aldolase and Sephadex G-25 (Pharmacia, Uppsala, Sweden) and charcoal (Baker Chemical Co., Phillipsburg, N.J., U.S.A.).

Cytosol preparation

The embryos were dissected midventrally to obtain the Müllerian duct (or other organs) for cytosol

preparation. All succeeding procedures were carried out at 0°C. The embryonic organs were pooled and washed with buffer twice and subsequently were minced finely with scissors. The minced tissue was homogenized in 5 vol. of buffer with a Teflon-glass homogenizer (Glenco, Houston, Tex., U.S.A.). Homogenates were centrifuged at 700g for 10 min at 0°C to obtain the crude nuclear pellets and the supernatants. The supernatants were again centrifuged at 105 000g at 0°C for 30 min in a Beckman model L5-65 ultracentrifuge in a rotor SW 50.1 with model 305527 adaptor. The resulting clear supernatants, designated 'cytosol', were adjusted to 4 mg of protein/ml with buffer.

Nuclear receptor preparation

Procedure 1. The crude nuclear pellet (from 150–200 Müllerian ducts), obtained by sedimentation as described above, was suspended in 10 vol. of 1.8M-sucrose prepared in buffer and then was centrifuged at 100 000g for 1 h. The resulting nuclear preparation was incubated for 1 h at 37°C with the cytosol from the 15-day embryonic left Müllerian duct; the cytosol used for incubation was prepared by preincubating it with 25 nM-[³H]oestradiol at 0°C for 1 h; the free oestradiol in the cytosol was removed by dextran-coated charcoal (with 0.25% charcoal and 0.025% dextran in the final volume). Nuclei were then sedimented and washed three times with 10 vol. of buffer. The nuclear [³H]oestradiol-receptor complex was extracted with buffer containing 0.5M-KCl.

Procedure 2. For this, 100 left Müllerian ducts were incubated in 2 ml of Hanks solution (Hanks & Wallace, 1949) containing 25 nM-[³H]oestradiol for 1 h at 37°C under O₂+CO₂ (95:5) with constant shaking. After incubation, Müllerian ducts were washed three times with 10 vol. of buffer before the nuclei were isolated from the tissue; the nuclear [³H]oestradiol-receptor complex was subsequently extracted by the procedure described above.

Hormone-binding assay

For the assay of the cytosol receptor, two series of assay tubes were used. The first series contained 500 µg of cytosol protein, equivalent to 8–10 Müllerian ducts, in 0.125 ml assay volume plus various concentrations of [³H]oestradiol as indicated. The second series contained the same quantities of cytosol protein and [³H]oestradiol plus a 50- or 100-fold excess of non-radioactive oestradiol or other steroid hormone. The two series of assay mixtures were incubated simultaneously at 0°C for 60 min, then 0.125 ml of 0.5% dextran-coated charcoal (with 0.05% dextran in buffer) was added to terminate the reaction; they were then stirred with a Vortex mixer three times in 10 min. The free steroids absorbed by char-

coal were removed by centrifugation at 3000g for 15 min in a Beckman model J-21B centrifuge. A 200 µl portion of the total of 250 µl of supernatant was removed for counting of radioactivity. The difference in the concentration of bound ligand in the first and the second series of incubations was considered as the specific binding.

Glycerol gradient

Cytosol (1.2 mg of protein) equivalent to 20 Müllerian ducts was centrifuged in a linear 5–35% (v/v) glycerol gradient (4.5 ml), prepared in buffer (with or without 0.5M-KCl), and centrifuged at 150 000g_{av} for 18 h at 0°C in a Beckman SW 50.1 rotor. Each gradient was fractionated with a Buchler piercer into 30 fractions (nine drops per fraction). Sedimentation markers (200 µg each of aldolase, bovine serum albumin and γ-globulin) were run in a separate gradient.

DNA-cellulose chromatography

DNA-cellulose was prepared from double-stranded DNA of 15-day chick embryos. Acid-washed cellulose (1 g; Munktells 410, Bio-Rad Laboratories, Richmond, Calif., U.S.A.) was mixed with 4 ml of DNA (2 mg/ml) with gentle stirring; then it was spread thinly over the inner surface of a beaker and dried overnight. The mixture was suspended in 20 ml of ethanol and placed under a u.v. light for 15 min with constant stirring. The ethanol was removed by filtration with suction on Whatman no. 2 paper, and DNA-cellulose was equilibrated with 0.15M-KCl in buffer until absorption of effluent at 260 nm was less than 0.03. Then 1–2 ml of [³H]oestradiol-labelled cytosol was allowed to settle into the cellulose for 15 min and subsequently was eluted with different concentrations of KCl in buffer.

DEAE-cellulose chromatography

DEAE-cellulose (Whatman DE-52) in buffer was packed into a column (1.5 cm × 10 cm) and equilibrated with 0.05M-KCl in buffer before use. Samples were applied in buffer, and the elution was carried out with a 100 ml linear gradient of 0.05–0.4M-KCl in buffer. Fractions (1.2 ml) were collected and the ionic strength of samples was measured with a CDM conductivity meter (Radiometer, Copenhagen, Denmark). The absorbance of the samples at E₂₈₀ was measured with a Pye-Unicam Sp. 1800 u.v. spectrophotometer, and 50 µl of each sample was assayed for radioactivity.

Isoelectric focusing

Isoelectric focusing was done in a small column (6 ml total volume) modified from the original design

recommended by Osterman (1970) and Mainwaring & Irving (1973). For analysis, oestradiol-binding protein (isolated from the DE-52 column) was adjusted to 5% (w/v) sucrose and loaded on top of a linear 10–60% (w/v) sucrose gradient (5ml) containing 1% (v/v) ampholytes (pH 3–11); it was formed in a Pyrex glass tube (0.8cm×15cm) [the bottom of the tube was previously plugged with 0.25ml of 10% acrylamide plus 0.2% *NN'*-methylenebisacrylamide, polymerized by adding 0.03ml of *NNN'*-tetramethylenediamine and 0.3ml of 10% (w/v) ammonium persulphate to 40ml of the acrylamide solution]. Four columns were run simultaneously with a single LKB model 3371E d.c. power supply in a Hoefer-DE 102 electrophoresis apparatus. The upper electrode (cathode) contained 1% (w/v) NaOH and the lower reservoir contained 1% (w/v) H₂SO₄. The run was carried out for 3–4h and reached a final field of 200V and 1.5mA. Then 25 fractions were collected from the bottom, and the pH of a portion of each fraction was measured at 0°C with a Digicord pH-meter (Photovolt, New York, N.Y., U.S.A.) and then assayed for radioactivity.

Fig. 1. Glycerol-gradient centrifugation of the hormone receptor

Portions (1.2mg) of cytosol protein (4mg of protein/ml) from Müllerian duct were treated individually as follows: ●, standard incubation (60min at 0°C with 10nM-[³H]-oestradiol); ○, standard incubation+20μg of ribonuclease in 5μl (this concentration of ribonuclease could hydrolyse 200μg of 28S chick oviduct RNA in 30min at 0°C); △, standard incubation in the presence of 0.5M-KCl (0.5M-KCl was present in the glycerol gradient). —, Standard incubation containing 50μg of Pronase; ····, standard incubation containing 1μM-diethylstilboestrol; ----, standard incubation in the presence of 1μM-oestradiol for competition. After incubation, the samples were mixed briefly with dextran-coated charcoal and centrifuged at 3000g for 15min to remove the unbound labelled steroid. Samples of the supernatants were layered on glycerol gradients for centrifugation (see the Materials and Methods section).

General procedures

Radioactivity was determined by adding 50–200μl samples to 4ml of scintillation fluid [6g of 2,5-diphenyloxazole, 0.15g of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre of toluene] into a mini counting vial (Rochester Scientific Co., Rochester, N.Y., U.S.A.). The radioactivity was determined at 60% efficiency in a Beckman model LS-250 scintillation spectrometer. Protein was determined by the procedure of Lowry *et al.* (1951) with bovine serum albumin (Sigma) as a standard. DNA was determined by the diphenylamine reaction (Giles & Myers, 1965).

Results

Glycerol-gradient analysis of the oestradiol receptor

The binding of [³H]oestradiol to the cytoplasmic and nuclear receptor (isolated from the left Müllerian duct of the 15-day female chick embryo) was examined by glycerol-gradient centrifugation. In the cytoplasm, two macromolecules were found to bind to oestradiol. To determine the sedimentation coefficient of the oestradiol-binding molecules in the embryonic organ, three purified protein standards (e.g. 4.5S bovine serum albumin, 7S bovine γ-globulin and 7.8S

Fig. 2. Glycerol-gradient centrifugation of nuclear hormone receptor

Nuclear receptor and [³H]oestradiol complex prepared by procedure 1 (●, 0.8mg of protein) or procedure 2 (○, 0.45mg of protein) (see the Materials and Methods section) was applied on a glycerol gradient (in the presence of 0.5M-KCl) for centrifugation.

Table 1. *Effect of various non-labelled steroids on binding of [³H]oestradiol to cytosol of Müllerian duct*

Cytosols were incubated for 1 h at 0°C with 10 nM-[³H]-oestradiol alone or in combination with either 0.5 or 1 μ M non-labelled steroid. The bound [³H]oestradiol was measured by the dextran-charcoal assay. Each value is the mean $\pm$ S.D. of three to four determinations.

Competing steroid added	Concn. of competing steroid (μ M)	[³ H]Oestradiol binding (% of control)
None		100
Cortisol	0.5	100 $\pm$ 0.50
	1	100 $\pm$ 0.40
Aldosterone	0.5	100 $\pm$ 0.40
	1	100 $\pm$ 0.35
Testosterone	0.5	97 $\pm$ 1.20
	1	97 $\pm$ 1.55
Progesterone	0.5	91 $\pm$ 1.44
	1	91 $\pm$ 1.56
Oestriol	0.5	23 $\pm$ 1.50
	1	22 $\pm$ 1.15
Oestrone	0.5	20 $\pm$ 1.20
	1	18 $\pm$ 1.05
Diethylstilboestrol	0.5	10 $\pm$ 1.00
	1	10 $\pm$ 1.50
Oestradiol	0.5	8 $\pm$ 0.83
	1	8 $\pm$ 0.85

rabbit liver aldolase) were centrifuged in glycerol gradient and their positions in the gradient were determined (Martin & Ames, 1961). With these standard proteins as markers, the sedimentation coefficients of the cytoplasmic oestradiol-receptor molecules were determined; their values were approx. 4.5S and 8S. At low ionic strength, [³H]oestradiol binds to the 8S and 4.5S cytoplasmic receptor, and the 8S receptor could be dissociated into a 4.5S unit in the presence of 0.5M-KCl. The binding of [³H]-oestradiol to form the 8S complex in cytosol was abolished by Pronase but not by ribonuclease (Fig. 1). The nuclear receptor-oestradiol complex, as isolated with high salt concentration by procedure 1 or 2, sediments in the 4.5S-6S region (Fig. 2).

Specificity of oestradiol binding

Table 1 and Fig. 3(a) show that, measured by the dextran-charcoal method, the binding of [³H]-oestradiol to Müllerian-duct cytosol was blocked 90-92% by a 100-fold excess of unlabelled diethylstilboestrol or oestradiol. No significant competition was observed by the same amount of aldosterone, cortisol, progesterone or testosterone. A Scatchard (Scatchard, 1949) plot based on the specific-binding data for oestradiol is a straight line (Fig. 3b), which indicates that within this range of steroid concentra-

Fig. 3. *Binding curve and Scatchard plot of Müllerian-duct cytosol*

(a) Specific cytoplasmic [³H]oestradiol binding as a function of added oestrogen. Cytosols were prepared from the Müllerian ducts of 15-day embryonic female chicks. ●, Total [³H]oestradiol binding; ▲, [³H]oestradiol plus 100-fold excess of oestradiol; ■, [³H]oestradiol plus 100-fold excess of diethylstilboestrol; the solid line represents the specific binding (difference between the total and the non-specific binding). (b) Scatchard plot derived from the specific binding.

tion a single class of binding sites is measured. The dissociation constant (K_d), calculated from the slope of the Scatchard plot, is $3.2 \times 10^{-9} M$. The concentration of binding sites calculated from the intercept and based on protein content is 1.57 nM/mg of cytosol protein. The estimation of the number of binding sites ($5.45 \text{ fmol}/10^6 \text{ cells}$) is based on the observation that the DNA content of the left Müllerian duct of the

Table 2. [^3H]Oestradiol binding to cytosol isolated from various embryonic organs

An equal amount (500 μg of protein/0.125 ml assay volume) of cytosol protein (4 mg of protein/ml) was assayed by incubating with (a) 25 nM- or (b) 0.1 μM -[^3H]oestradiol for 60 min at 0°C as in the standardized assay technique. All data presented were corrected by subtracting non-specific binding obtained in the presence of 100-fold excess of unlabelled oestradiol. Each value is the mean $\pm$ S.D. of four determinations.

Source	Specific binding of [^3H]oestradiol to cytosol (nm/mg of protein)	
	(a)	(b)
Müllerian duct	1.420 $\pm$ 0.05	1.420 $\pm$ 0.04
Ovary	0.140 $\pm$ 0.02	0.165 $\pm$ 0.02
Skeletal muscle	0.034 $\pm$ 0.015	0.033 $\pm$ 0.013
Liver	0.033 $\pm$ 0.015	0.034 $\pm$ 0.02
Brain	0.039 $\pm$ 0.01	0.032 $\pm$ 0.015
Heart	Not detectable	Not detectable

Fig. 4. Binding of Müllerian-duct cytosol-oestradiol complexes to chick DNA-cellulose

Cytosol protein (8 mg) in 2 ml of buffer was incubated with 25 nM-[^3H]oestradiol for 60 min at 0°C and the free oestradiol was removed by adding an equal volume of dextran-charcoal as described in the Materials and Methods section. The cytosol-[^3H]oestradiol complex (2 ml) was settled on to the DNA-cellulose column for 15 min, and then it was eluted with different concentrations of KCl into fifty fractions (1 ml/fraction). A portion of this preparation containing 9.5×10^4 c.p.m. was eluted (●); 3 mg of whole serum protein (15-day embryos) was complexed with [^3H]oestradiol under the same conditions as described for cytosol protein (○); pure cellulose was prepared for chromatography without DNA (▲); the dashed line represents the free [^3H]oestradiol (about 1.0×10^5 c.p.m.) eluted.

15-day embryonic female is $11.5 \pm 0.9 \mu\text{g}/\text{duct}$ and the knowledge that the average DNA content for 10^6 embryonic cells is 2.5 μg (Sober, 1968). [^3H]Oestradiol binding to the cytosol of various organs of the female embryo at the same age is presented in Table 2. The results indicate that oestrogen selectively interacts with its target organ. Trace amounts of binding were observed in the ovary, skeletal muscle, brain and liver, but no binding was detectable in the heart.

Interaction of hormone-receptor complex with host DNA

Left-Müllerian-duct cytosol and whole serum protein were prepared from 15-day female chick embryos. The association between host DNA and the cytosol-[^3H]oestradiol complex was determined. The elution profiles from DNA-cellulose are summarized in Fig. 4 and show that 9% of the Müllerian-duct oestradiol-receptor complex was bound to the DNA-cellulose column in the presence of 0.14 M-KCl, and could be eluted from the DNA-cellulose column by 0.5 M-KCl. Under the same experimental conditions, no detectable binding was observed when comparable amounts of whole serum protein plus [^3H]oestradiol were added. No retention of radioactivity was observed in the control experiments when [^3H]oestradiol was applied to a DNA-cellulose column or when the cytosol-[^3H]oestradiol complex was placed on a column of pure cellulose alone.

DEAE-cellulose chromatography

The complex of cytosol and [^3H]oestradiol was chromatographed on a DEAE-cellulose column (the elution profile is shown in Fig. 5a). Five major peaks were detected by measuring the E_{280} . Four peaks (I, II, III and IV) were found to be labelled with [^3H]oestradiol. Glycerol-gradient analysis (Fig. 6) showed that radioactivity corresponding to peaks II and III sedimented as a single 4.5 S peak and that peak IV sedimented as an 8 S peak with some contamination of 4.5 S material. No distinct sedimentation pattern was observed for peak I (results not shown). Further characterization of peaks III and IV by isoelectric focusing (Fig. 5b) indicated that the pI for peak III is 6.9, whereas that of peak IV is mainly 5.8, with a minor component with pI 6.9. The pI of the 8 S receptor is similar to that of the 8 S oestradiol receptor observed in the calf uterus (DeSombre *et al.*, 1968) and to the 5 α -dihydrotestosterone receptor in the rat prostate gland (Mainwaring & Irving, 1973).

Discussion

These experiments demonstrate the existence of an oestradiol-binding macromolecule in the Müllerian ducts of the 15-day embryonic female chick. The

Fig. 5. Elution profile of the $[^3\text{H}]$ oestradiol-receptor fraction from a DEAE-cellulose column

(a) Cytosol protein (12mg) in 2ml of buffer was complexed with $[^3\text{H}]$ oestradiol as described in Fig. 3 and eluted through the column by a linear 0.05–0.4M-KCl gradient with the flow rate adjusted to 0.6ml/min. A total of 75 fractions (1.2ml/fraction) was collected. $\circ$, Radioactivity; $\bullet$, E_{280} ; $\blacktriangle$, [KCl]. (b) Isoelectric-focusing patterns of two radioactive fractions from DEAE-cellulose. Receptor protein (200 μg) was pooled from the peak areas of fraction III and of fraction IV and used for focusing as detailed in the Materials and Methods section. $\bullet$, Fraction III; $\circ$, fraction IV; $\blacktriangle$, pH.

Fig. 6. Sedimentation pattern of three radioactive fractions from the DEAE-cellulose column in 5–35% glycerol gradient. Protein (450 μg) was pooled from the peak area of fraction II (Δ), III ($\bullet$) or IV ($\circ$) and mixed with dextran-charcoal (see the Materials and Methods section) to remove the unbound labelled steroid, and then centrifuged on the gradient (see the Materials and Methods section). Thirty fractions (0.15ml/fraction) were collected, and a portion (0.1 ml) of each fraction was added with scintillation fluid for radioactivity determination.

macromolecule's characteristics are similar to known sex-steroid receptors of various target tissues (Toft & Gorski, 1966; O'Malley *et al.*, 1970; Cox *et al.*, 1971; Jung & Baulieu, 1972). The observed selective binding of oestradiol to the cytosols of Müllerian ducts is consistent with the observation by Jonsson & Terenius (1965) of the broody-chick oviduct. The results are also consistent with previous observations of organ-specific binding of steroid hormones, e.g. the uterus, vagina, hypothalamus and hypophysis for oestradiol (Jensen & Jacobson, 1960, 1962; Kato & Villet, 1967) and the seminal vesicles and prostate gland for testosterone (Butenandt *et al.*, 1960; Harding & Samuels, 1962).

The steroid specificity of the Müllerian-duct receptor confirms some previous observations that the oestrogen receptor binds to oestrogen but not to other steroid hormones, e.g. cortisol, progesterone and testosterone (Korenman, 1969; Notides, 1970). The dissociation constant (K_d) measured at 0°C showed that the oestradiol-binding affinity of the Müllerian-duct cytosol receptor is $3.2 \times 10^{-9} \text{ M}$, which is equivalent to the K_d value ($3.0 \times 10^{-9} \text{ M}$) of the oestradiol receptor obtained in the newborn-rat uterus (Clark & Gorski, 1970).

Gradient centrifugation after the addition of excess of unlabelled diethylstilboestrol (or oestradiol) to the Müllerian-duct cytosol showed, however, the

presence of specific low-capacity binding in the 4.5S area. This indicated that the specific receptors for oestradiol occurred in both the 8S and 4.5S areas of the gradient. Yet the possible existence of a certain amount of serum albumin, which has high capacity for [³H]oestradiol binding (Peck *et al.*, 1973), sedimenting in the 4.5S area should not be ruled out. Glycerol-gradient analysis showed that in the presence of 0.5M-KCl, the 8S hormone-receptor complex dissociated to the 4.5S form, which is in contrast with the observation that the cytoplasmic 10S oestradiol receptor of the immature chick oviduct is not dissociated by 0.5M-KCl (Cox *et al.*, 1971). Further, a 4.5S-6S receptor was extracted from the nuclear fraction. This observation is comparable with the finding in rat uterus, where 5S-6S nuclear receptor proteins have been characterized (Jensen *et al.*, 1967; Giannopoulos & Gorski, 1971b). The sedimentation pattern of the embryonic 8S and 4.5S oestradiol-receptor complexes is similar to that of several known steroid receptors, e.g. the oestrogen receptor of the uterus (Erdos, 1968; Korenman & Rao, 1968; Puca *et al.*, 1972), the 5 α -dihydrotestosterone receptor of the prostate gland (Mainwaring, 1969; Mainwaring & Irving, 1973), and the cortisol receptor in the embryonic chick retina and foetal rabbit tissues (Chader *et al.*, 1972; Giannopoulos *et al.*, 1974). However, the pattern differs from that of the oestradiol receptor of the neonatal rat brain and from the cortisol receptor of the embryonic chick liver; in both of these instances, only one species of 4S macromolecule was observed (Plapinger *et al.*, 1973; Tu & Moudrianakis, 1973).

The observed 9% of the hormone-receptor complex bound to DNA is comparable with the value obtained by Clemens & Kleinsmith (1972) in the rat uterine system. Our value is lower than the 30% obtained by Mainwaring & Irving (1973) in the prostate gland. This aspect of receptor interaction with DNA and its physiological meaning is still open for speculation and remains to be investigated.

When Müllerian-duct cytosol was eluted through a DEAE-cellulose column by a linear KCl gradient, five distinct protein components were obtained; four of them showed oestradiol-binding capacity, and this chromatographic pattern is highly reproducible. We have at present no adequate explanation for this observed multiplicity of oestrogen binding in the Müllerian-duct cytosol. However, the observation may reflect the following possibilities. (a) The embryonic organ is actively participating in utilizing the oestrogen for the purpose of growth and differentiation. One or two of the observed receptor proteins may be involved in the binding of the oestrogen metabolites. (b) It is possible that there are receptors for oestrone and oestriol existing in the Müllerian-duct cytosol, and these receptors could bind oestradiol.

The following characteristic properties of this hormone-binding macromolecule distinguish it from the other non-specific oestradiol-binding molecules that possibly exist in the Müllerian duct: (a) its protein nature and relatively acidic isoelectric point, (b) its binding affinity to DNA-cellulose, (c) its [³H]oestradiol-saturable character, and (d) its steroid- and organ-specificity for oestradiol binding. These properties suggest that it is a constituent of the embryonic sex organ. The ontogeny of this oestradiol receptor in the developing organ is explored in the following paper (Teng & Teng, 1975).

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