Synthesis of apoprotein B of very low density lipoprotein in embryonic-chick liver

Development of responsiveness of oestrogen

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The influence of exogenous oestrogen on the rate of synthesis of the apoprotein B of very-low-density lipoprotein by embryonic chick liver has been examined at various stages of development. Significant synthesis of apoprotein B was found in the absence of hormone treatment as early as day 6 of egg incubation. This basal level of apoprotein B synthesis varied from 2.5–6% of total protein synthesis. Embryos at day 10 or earlier treated with oestradiol exhibited no increase in the basal level of apoprotein B synthesis. Embryos at day 11 responded to oestradiol slightly and, from days 12 to 20, oestrogen treatment raised the relative rate of apoprotein B synthesis significantly above basal values: the maximal rate was about 16% of total protein synthesis. The anti-oestrogen tamoxifen citrate abolished the hormone-induced increase in apoprotein B synthesis and was not itself oestrogenic. The basal level of apoprotein B production was not sensitive to tamoxifen citrate, either in relatively early or in later stages of development. The basal level of apoprotein B synthesis, therefore, is oestrogen-independent and under developmental control distinct from the hormone-sensitive synthesis. The ontogeny of oestrogen-responsiveness of apoprotein B production appears to parallel the acquisition of the hepatic oestrogen-receptor system [Lazier (1978) *Biochem. J.* 174, 143–152].

The advantages of using oestrogen-induced vitellogenesis by oviparous vertebrate liver as a model system for studying the mechanism of sex steroid hormone action have already been well-documented (Ryffel, 1978; Tata & Smith, 1979). However, in chick liver, oestrogen stimulates the synthesis of other proteins besides vitellogenin; transferrin (Lee *et al.*, 1978), riboflavin-binding protein (Murthy & Adiga, 1978), and VLDL apoproteins (Luskey *et al.*, 1974; Chan *et al.*, 1976, 1978; Williams, 1979). The study of multiple markers for oestrogen-responsive gene expression adds another dimension to the model, and permits investigation of the degree of co-ordination of the responses under a variety of different conditions, such as, for example, during embryonic development and differentiation. This could lead to understanding of common and unique factors involved in

the establishment and maintenance of hormonal regulation of the different genes.

In this study we examine in embryonic-chick liver the ontogeny of the response to oestrogen of the apoprotein B of VLDL. This very large protein makes up about half of the protein present in plasma VLDL particles. The apparent mol.wt. on SDS/polyacrylamide-gel electrophoresis is 350 000. It is the only high-molecular-weight apoprotein seen in VLDL, provided that precautions against proteolytic degradation are taken during preparation (Williams, 1979). Most of the remaining protein in the chicken VLDL is apoprotein II, a small dimer consisting of two identical subunits of 82 amino acids each (Jackson *et al.*, 1977). Synthesis of both of the major VLDL apoproteins is oestrogen-controlled. There is, however, a significant hepatic synthesis and serum concentration of both apoproteins in roosters that have not been treated with oestrogen (Chan *et al.*, 1978; Williams *et al.*, 1979; Capony & Williams, 1980).

Vitellogenin synthesis in embryonic-chick liver becomes inducible by oestrogen during days 13–15

Abbreviations used; VLDL, very-low-density lipoprotein; SDS, sodium dodecyl sulphate.

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of egg incubation (Lazier, 1978). In contrast with the VLDL apoproteins, there is no hepatic vitellogenin synthesis or accumulation of vitellogenin in serum in untreated embryos or in roosters (Deeley *et al.*, 1977; Lazier, 1978; Chan *et al.*, 1978). The present study shows that apoprotein B of VLDL becomes oestrogen-inducible somewhat earlier than does vitellogenin; some response can be seen at about day 11 of development. In addition, the basal level of apoprotein B synthesis appears to be oestrogen-independent as suggested by its insensitivity to anti-oestrogen treatment and by its distinctive ontogeny. Significant basal synthesis of apoprotein B can be detected as early as day 6 of egg incubation.

Materials and methods

Materials

L-[4,5- ^3H (N)]Leucine of sp. radioactivities 51.6 and 45.7 Ci/mmol and ^{14}C -labelled bovine serum albumin of sp. radioactivity 13.2 $\mu\text{Ci}/\text{mg}$ were from New England Nuclear, Montreal, Quebec, Canada as were Omnifluor and Protosol. Anti-(bovine serum albumin) antibody was from Miles-Yeda Research Products, Rehovot, Israel. All marker proteins for SDS/polyacrylamide-gel electrophoresis were from Pharmacia (Montreal, Quebec, Canada) except for *Escherichia coli* RNA polymerase and bovine serum albumin, which were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Oestradiol-17 β , L-leucine and phenylmethanesulphonyl fluoride were also from Sigma. All other chemicals were of reagent grade.

Animals and injections

Fertile white Leghorn eggs, obtained from Cooks' Hatchery (Truro, Nova Scotia, Canada) were kept in a humidified incubator at 38°C. Eggs were injected with 0.1 ml of propylene glycol solution of oestradiol-17 β or tamoxifen citrate or with the propylene glycol vehicle alone just underneath the air sac membrane. The doses used are indicated in the text. At the end of the exposure period (usually 19 h) embryos were killed and the livers or other tissues were used for determination of apoprotein B synthesis *in vitro*.

Determination of the relative rate of synthesis of apoprotein B

Embryonic liver or other tissue as indicated was incubated for 1 h at 40°C in [^3H]leucine-containing medium exactly as described in detail for rooster-liver slices by Capony & Williams (1980). The incubation conditions gave linear incorporation of [^3H]leucine into tissue protein and negligible release of labelled apoprotein B into the medium. Tissue (20 mg) was incubated in 100 μl of medium; for liver,

sufficient tissue was derived from a single embryo at day 12 or later for one incubation mixture, but at early stages two or three livers were pooled. After incubation, the liver pieces were cooled to 2°C, washed and homogenized in buffer containing 1% (w/v) Triton X-100 and 200 μg of phenylmethanesulphonyl fluoride/ml. The homogenate was centrifuged at 100 000 g for 1 h and the supernatant was assayed for immunoreactive ^3H -labelled apoprotein B and for total [^3H]leucine incorporation into trichloroacetic acid-insoluble protein.

Monospecific rabbit antiserum against apoprotein B, prepared and characterized by Williams (1979), was used for the direct immunoprecipitation assay for labelled apoprotein B in the tissue supernatants. Validation and detailed quantification of this assay is discussed by Capony & Williams (1980). The incubation mixture contained 10 μl of the embryonic supernatant, 20 μl of antiserum and 20 μl of VLDL carrier solution. Incubation, sedimentation and washing of the immunoprecipitates, dissolution of the precipitates in Protosol and counting by liquid scintillation was exactly as detailed by Capony & Williams (1980). Precipitin curves with a range of carrier VLDL concentrations were carried out to ensure that optimal immunoprecipitation in the region of antibody excess was obtained.

Non-specific immunoprecipitation was determined by immunoprecipitation of bovine serum albumin by anti-(bovine serum albumin) antibody in the presence of 10 μl of liver supernatant. The proportions of antigen to antibody were adjusted so that the weight of immunoprecipitate was similar to that obtained with anti-(apoprotein B). This system gave rather higher values for non-specific immunoprecipitation (about 1% of total trichloroacetic acid-precipitable counts in 10 μl of supernatant) than a system in which preimmune serum replaced anti-(apoprotein B). All immunoprecipitation results have been corrected by using the bovine serum albumin/anti-(bovine serum albumin) method.

Total [^3H]leucine incorporation into supernatant protein was determined for each supernatant by precipitation with trichloroacetic acid as described by Capony & Williams (1980). The results for the rates of apoprotein B synthesis are thus expressed in each case as a percentage of total supernatant protein synthesis.

SDS/polyacrylamide-gel electrophoresis

For electrophoresis in gels containing 5% (w/v) acrylamide, samples and gels were prepared as described by Williams (1979) for system A except that tube gels (about 9 cm long) were used and a stacking gel containing 3% (w/v) acrylamide was employed. The running buffer used was that described by Laemmli (1970). Gels were run for 3–4 h

at room temperature at a current of 3 mA/tube. Gels were either fixed and stained overnight in 50% (w/v) trichloroacetic acid containing 0.1% Coomassie Blue and destained by diffusion in 7% (v/v) acetic acid or were frozen with solid CO₂ and cut into 0.3 cm slices with a Mickle gel slicer. Gel slices were allowed to swell at room temperature for about 3 days in 10 ml of scintillation fluid containing Omnifluor (0.8%, w/v) and Protosol (3%, v/v) in toluene before being counted for radioactivity in a Nuclear Chicago Isocap 300 liquid-scintillation spectrometer with an efficiency of about 45%.

Protein determinations

The protein content of immunoprecipitates was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as standard, except that 1% (w/v) SDS was included in the assay to prevent any lipid present from making the assay cloudy.

Results

Time course and dose-response of the oestrogenic induction of apoprotein B in embryonic-chick liver

Embryonated eggs at day 16 of development were injected with a large dose of oestradiol (2.5 mg) or with vehicle alone and the capacity of the isolated liver pieces to synthesize apoprotein B was measured at various times thereafter. Fig. 1 shows that the basal level of synthesis was steady, accounting for about 3.8% of the total protein synthesis. By 5 h after oestradiol injection synthesis of apoprotein B was significantly increased, and by 19 h made up 15% of total protein synthesis. Even after 48 h the response was still pronounced. The 19 h time was used for subsequent experiments.

Fig. 2 summarizes the dose-response characteristics for liver from 17-day embryonated eggs that had been injected with oestradiol 19 h previously. Livers from embryos not given oestradiol exhibited a rate of apoprotein B synthesis of about 3.5% of total protein synthesis. This increased to about 14% with saturating doses of the hormone. Oestradiol doses of 1.25 mg and 2.5 mg per egg elicit a maximal response. These doses are similar to those reported earlier for maximal stimulation of the embryonic-liver nuclear oestrogen receptor (Gschwendt, 1977a), and for induction of vitellogenin (Lazier, 1978). The amount of the hormone that actually reaches the embryo is not known.

Ontogeny of synthetic capacity for apoprotein B and of oestrogen inducibility

The elevation of the relative rate of apoprotein B synthesis elicited in chick liver by oestradiol injection was used to probe the responsiveness of liver tissue to the hormone over a large part of the embryonic development period. To maximize the

sensitivity, eggs were injected with 2.5 mg of oestradiol 19 h before use (cf. Figs. 1 and 2). All control embryos received propylene glycol only, again 19 h before use, except for 6-day embryos, which received no injection. Propylene glycol was toxic to these very young embryos, but at later stages of development no toxic effects were evident. Furthermore, propylene glycol itself was shown to have no effect on apoprotein B synthesis in embryos at the 8-day stage. Treatment of the 8-day embryos 19 h earlier with propylene glycol resulted in a relative rate of apoprotein B synthesis of $4.0 \pm 0.2\%$, whereas no treatment gave a rate of $4.1 \pm 0.2\%$.

Fig. 3 illustrates the results of the ontogeny study. From day 6 to day 20, a basal level of apoprotein B synthesis was observed in control liver. The rate of this synthesis varied from 2.5 to 6.0% of total protein production. In liver of embryos up to day 10, oestradiol was unable to induce increases in apoprotein B synthesis above basal levels. At day 11, a small response to hormone was apparent and from day 12 onwards a pronounced response occurred.

Fig. 1. *Time course of the response of apoprotein B synthesis to oestradiol*

Embryonated eggs (16-day) were injected with either 2.5 mg of oestradiol in 0.1 ml of propylene glycol (●) or 0.1 ml of propylene glycol only (○). At 5, 19 and 48 h after injection, livers were removed and were used to prepare supernatants and were assayed for the relative rate of apoprotein B synthesis as described in the Materials and methods section. Each point is the mean $\pm$ s.d. for duplicate determinations of three separate supernatants.

Fig. 2. Dose-response for oestrogen induction of apoprotein B synthesis

Chick embryos (17-day) from eggs that had been injected with 0–2.5 mg of oestradiol 19 h earlier were killed, livers were removed and supernatants prepared and assayed for the relative rate of apoprotein B synthesis as described in the Materials and methods section. Each point is the mean $\pm$ s.d. for three separate supernatants.

Fig. 3. Ontogeny of apoprotein B synthesis and oestrogen stimulation in embryonic-chick liver

Embryonated eggs at different stages of development were injected with 2.5 mg of oestradiol in propylene glycol (●) or with 0.1 ml of propylene glycol (○) 19 h before killing or, in the case of 6-day embryos, were untreated. Livers were incubated, supernatants prepared and the relative rate of apoprotein B synthesis was measured as described in the Materials and methods section. Each point is the mean $\pm$ s.d. for duplicate determinations of three separate supernatants.

The variation in the basal level of synthesis complicates efforts to determine precisely at what stage oestrogen can raise apoprotein B synthesis above control values, but the day 10–11 period appears to be a crucial stage in the acquisition of hormone responsiveness in the synthesis of this protein.

The overall rates of total protein synthesis, although quite variable, exhibited no significant changes in the course of development in livers from either oestrogen-treated or control embryos (results not shown). Similar observations were made earlier with somewhat different liver incubation conditions (Lazier, 1978). The variability in the total protein synthesis seen is most likely due to differences in [3 H]leucine uptake by the tissue slices of somewhat different geometry, and expression of the rate of apoprotein B synthesis relative to total protein synthesis serves to minimize these differences.

SDS/polyacrylamide-gel electrophoresis of immunoprecipitates

In order to check the identity of the 3 H-labelled material being precipitated by anti-(apoprotein B) serum, immunoprecipitates of liver supernatants at

selected stages were analysed by SDS/5% (w/v) polyacrylamide-gel electrophoresis. 14 C-labelled bovine serum albumin was run on the same gel as each immunoprecipitate and acted as an internal marker. Other marker proteins, including authentic apoprotein B, were run on similar gels simultaneously.

Figs. 4(a) and 4(b) illustrate that immunoprecipitates with anti-(apoprotein B) serum of 6- and 8-day embryo liver supernatants contain a 3 H-labelled band co-migrating with authentic apoprotein B. Oestradiol does not increase the size of this peak above control values. Electrophoresis of immunoprecipitates from 20-day embryo liver supernatants (Fig. 4c) demonstrates the pronounced effect of oestradiol on apoprotein B synthesis at this stage. It is obvious in all five gel profiles that there is a smaller peak of radioactivity at the dye-front position. About half of the 3 H-labelled material at the dye front can be accounted for by non-specific immunoprecipitation as measured by the bovine serum albumin/anti-(bovine serum albumin) method and the remainder is probably a mixture of apoprotein B

breakdown products and incomplete nascent chains (results not shown). The electrophoresis results confirm that the immunoprecipitation technique is specific for apoprotein B.

Fig. 4. SDS/polyacrylamide-gel electrophoresis of anti-(apoprotein B) immunoprecipitates from embryonic-liver supernatants

Supernatants from embryonic liver were prepared and analysed as described in the Materials and methods section. (a) Electrophoresis in 5% (w/v) polyacrylamide gels of SDS-treated immunoprecipitate from 10 μ l of supernatant from 6-day embryonic liver. Incorporation of [3 H]leucine into total protein was 1.54×10^5 c.p.m./10 μ l of supernatant and recovery on the gel was 95%. (b) Electrophoresis in 5% (w/v) polyacrylamide gels of SDS-treated immunoprecipitates from 5 μ l of liver supernatant from 8-day control (O) and oestradiol-treated (●) embryos. Incorporation of [3 H]leucine into total protein was 1.77×10^5 and 1.65×10^5 c.p.m./10 μ l of supernatant and recoveries on the gels were 76% and 84% respectively. (c) Electrophoresis in 5% (w/v) polyacrylamide gels of SDS-treated immunoprecipitates from 5 μ l of liver supernatants from 20-day control (O) and oestradiol-treated (●) embryos. Incorporation of [3 H]leucine into total protein was 0.80×10^5 and 1.16×10^5 c.p.m./10 μ l of supernatant and recoveries on the gels were 85 and 87% respectively. The identity and molecular weights of the marker proteins were as follows: V, apoprotein B (mol.wt. 350 000); T, thyroglobulin (mol.wt. 330 000); R, *E. coli* RNA polymerase β' subunit (mol.wt. 165 000); P, phosphorylase (mol.wt. 94 000); B, bovine serum albumin (mol.wt. 68 000); O, ovalbumin (mol. wt. 43 000). D indicates the position of the Bromophenol Blue dye front.

Effect of the anti-oestrogen tamoxifen citrate on apoprotein B synthesis by embryonic liver

Table 1 summarizes the effects of the anti-oestrogen tamoxifen citrate on the oestradiol-induced and basal levels of synthesis of apoprotein B. In 18-day embryo liver it is apparent that tamoxifen, given at twice the dose of oestradiol, completely abolishes oestradiol-induced apoprotein B synthesis without affecting the basal level of synthesis of the protein. No induction of apoprotein B is apparent on treatment with tamoxifen alone, at either day 10 or day 18. These data support the contention that tamoxifen acts as a pure anti-oestrogen in the chicken (Sutherland *et al.*, 1977; Capony & Williams, 1980).

The finding that the basal level of apoprotein B synthesis is unaffected by tamoxifen and that the basal level of synthesis occurs in liver before the tissue becomes responsive to exogenous oestradiol strongly suggests that this basal level of synthesis is oestrogen-independent.

Tissue specificity of embryonic apoprotein B synthesis

In view of the apparent oestrogen-independent synthesis of apoprotein B in embryonic-chick liver, we used the same methods to test other embryonic tissues for their ability to make apoprotein B. Table 2 shows that untreated 9-day embryo gizzard, heart and brain apparently produce apoprotein B at low levels, but when these tissue supernatant immunoprecipitates were analysed on SDS/5% (w/v) polyacrylamide gels, no 3 H-labelled apoprotein B peak was seen (results not shown). Similarly, when 15-day embryos, pre-treated with oestradiol or propylene glycol, were used to provide brain and gizzard or small intestine supernatants, these tissues appeared to synthesize apoprotein B at low rates, with oestradiol having no effect. These low values were found to be due to non-specific immunoprecipitation by using SDS/5% (w/v) polyacrylamide-gel electrophoresis. It was therefore concluded that no detectable apoprotein B synthesis occurs in embryonic heart, gastrointestinal tract or brain tissue.

Discussion

The existence of a basal level of synthesis of apoprotein B from early stages of hepatic development has a number of interesting implications. For two reasons, this synthesis appears to be oestrogen-independent. First, the basal level of synthesis is present in 6–10 day embryonic livers, at which time the tissue is unresponsive to exogenous oestrogen. Second, the basal level of synthesis in young or older embryos is unaffected by tamoxifen citrate at doses that completely abolish oestrogen-induced increases

Table 1. *Effect of tamoxifen citrate on synthesis of apoprotein B*

Embryos (10 to 18 day) were killed 19 h after injection of eggs with oestradiol, tamoxifen citrate or propylene glycol as indicated. Livers were used to prepare supernatants which were assayed for apoprotein B synthesis as described in the Materials and methods section. Each set of values is the mean $\pm$ s.d. for duplicate determinations on three separate supernatants.

Age of embryo at injection (days)	Embryo treatment	Synthesis of apoprotein B (% of total protein production)
10	Propylene glycol	5.6 $\pm$ 0.4
	Tamoxifen (0.5 mg)	5.3 $\pm$ 0.7
	Tamoxifen (1.0 mg)	5.2 $\pm$ 0.6
	Tamoxifen (1.5 mg)	6.3 $\pm$ 1.0
18	Oestradiol (0.5 mg)	13.0 $\pm$ 2.9
	Oestradiol (0.5 mg) + tamoxifen (0.5 mg)	3.7 $\pm$ 0.6
	Oestradiol (0.5 mg) + tamoxifen (1.0 mg)	3.1 $\pm$ 0.4
	Propylene glycol	3.8 $\pm$ 0.2
	Tamoxifen (0.5 mg)	4.3 $\pm$ 0.8
	Tamoxifen (1.0 mg)	3.5 $\pm$ 0.6

Table 2. *Tissue specificity of synthesis of apoprotein B*

Embryos (9-day from untreated eggs, or 15-day from eggs that had been injected with 2.5 mg of oestradiol or 0.1 ml of propylene glycol 19 h earlier) were killed and tissues were incubated and supernatants prepared as described for liver in the Materials and methods section. Supernatants were assayed for the relative rate of apoprotein B synthesis. Each value is the mean $\pm$ s.d. for duplicate determinations on three separate supernatants.

Age of embryos (days)	Treatment	Tissue	Synthesis of apoprotein B (% of total protein production)
9	None	Gizzard	0.3 $\pm$ 0.0
	None	Heart	0.3 $\pm$ 0.1
	None	Brain	0.8 $\pm$ 0.1
	None	Liver	6.4 $\pm$ 0.8
15	Propylene glycol	Gizzard	1.5 $\pm$ 0.3
	Oestradiol		1.6 $\pm$ 0.1
	Propylene glycol	Small intestine	0.2 $\pm$ 0.1
	Oestradiol		0.2 $\pm$ 0.1
	Propylene glycol	Brain	0.8 $\pm$ 0.1
	Oestradiol		0.7 $\pm$ 0.2
	Propylene glycol	Liver	3.8 $\pm$ 0.9
	Oestradiol		13.8 $\pm$ 0.1

in apoprotein B synthesis in older oestrogen-responsive embryos. There are therefore two major levels of control of apoprotein B gene expression and these exhibit different ontogeny.

In keeping with current ideas for the mechanism of oestrogen action, one could postulate that the apoprotein B gene is transcribed at a certain rate in the absence of oestrogen, and that the hormone, through receptor-chromatin interaction, increases the rate of expression of the same gene (Gorski & Gannon, 1976; Chan & O'Malley, 1976). On the other hand, the two levels of control of apoprotein B synthesis could reflect the presence of two classes of apoprotein B genes, one of which is under oestrogenic regulation and the other under a different mode of control. The possibility of multiple genes is worthy of consideration due to the recent demonstration of

several classes of oestrogen-responsive ovalbumin genes in chicken (Royal *et al.*, 1979), and vitellogenin genes in both chicken (Wang & Williams, 1980) and *Xenopus laevis* (Wahli *et al.*, 1979). Furthermore, the *Xenopus* genome may contain some vitellogenin genes that are not hormone-sensitive (J. R. Tata, personal communication). The question of gene heterogeneity for apoprotein B has been addressed to a certain extent at the level of the protein. Capony & Williams (1980) found no difference in the peptide 'maps' of apoprotein B made under basal conditions or after oestrogen induction. Apoprotein B from the two sources exhibited immunological cross-reactivity and similar electrophoretic mobility. These comparisons suggest, but do not prove, identity; further protein-structural studies are required.

Modern nucleic acid technology makes it possible to approach the question of apoprotein B heterogeneity and gene multiplicity at the level of genome organization and mRNA production. Cloned cDNA probes have recently been made for rooster apoprotein II mRNA sequences (Chan *et al.*, 1979; Wieringa *et al.*, 1979), for rooster vitellogenin (Ohno *et al.*, 1980) and for another, as yet identified, oestrogen-induced mRNA (King *et al.*, 1979). cDNA probes to apoprotein B mRNA from oestrogen-treated and control chicks could thus be used to investigate the question of whether or not the oestrogen-dependent and -independent control mechanisms for apoprotein B gene expression influence different classes of genes.

The basal rate of apoprotein B synthesis in the chick embryo liver tends to be higher than that observed in liver from roosters (Capony & Williams, 1980). This may reflect a requirement for more lipid transport in the developmental period than after hatching, but the mechanism of regulation of this kind of variation is unknown. Oestrogen-inducible proteins in avian liver differ in whether or not detectable synthesis of the protein is observed in the absence of the hormone. Basal synthesis has been reported for apoprotein II (Chan *et al.*, 1976, 1978) and for transferrin (Lee *et al.*, 1978). On the other hand, no basal synthesis has been found for vitellogenin in embryonic-chick (Lazier, 1978) or rooster (Deeley *et al.*, 1977) livers, or for riboflavin-binding protein (Claggett *et al.*, 1970; Murthy & Adiga, 1978) or for the oestrogen-induced protein encoded in sequences present in the cDNA clone E18 from rooster liver (King *et al.*, 1979). On this basis, oestrogen-controlled specific protein synthesis in avian liver falls into two general classes, characterized by the presence or absence of significant constitutive production of the protein in question.

The oestrogen-controlled hepatic proteins show some differences with respect to the kinetics of induction (Williams *et al.*, 1979). In addition, there are distinctive differences in the embryonic development of responsiveness to oestrogen (Lazier, 1978). Whereas apoprotein B is clearly oestrogen-inducible by day 11, vitellogenin synthesis cannot be evoked until days 13–15. It is of obvious interest to learn what factors endow specific genes with hormone sensitivity.

In the case of apoprotein B, inducibility by oestrogen bears a fairly close temporal correlation with the ontogenesis of the hepatic oestrogen receptor system (Gschwendt, 1977a,b; Lazier, 1978, 1979, 1980). Low amounts of salt-soluble receptor are normally present in embryonic liver from day 10. Injection of eggs with oestradiol on day 8 does not significantly affect the nuclear receptor concentration measured on day 10, but injection of the hormone on day 10 results in a sizeable increase in

nuclear receptor concentration on day 12. This is taken to reflect development of the receptor-translocation and nuclear-accumulation mechanisms between days 10 and 12. The apparent delay in inducibility of vitellogenin may indicate a requirement for specialized functions distinct from those sufficient for apoprotein B synthesis. In chick embryo Müllerian duct, the mechanism for translocation of the oestrogen receptor to the nucleus also appears to be present by day 12 (Teng & Teng, 1975, 1976). However, in this case, the fraction of nuclear receptor that complexes tightly with chromatin is not maximal until about day 15, and this correlates well with a period of increased inducibility of ovalbumin mRNA (Teng & Teng, 1978; Andrews & Teng, 1979). More detailed knowledge of the nature of the nuclear elements with which the receptor interacts, along with better understanding of the properties of receptor, is a clear priority for further analysis of the molecular basis of the development of responsiveness to oestrogen.

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