

# Progesterone Receptor in the Chick Oviduct: an Immunohistochemical Study with Antibodies to Distinct Receptor Components

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**ABSTRACT** We performed immunohistochemical studies of chicken oviduct after different fixation procedures, by using antibodies against the progesterone receptor: polyclonal antibodies IgG-G3 against the "8S" form (an oligomere containing progesterone-binding and nonprogesterone-binding units), polyclonal antibodies IgG-RB against the progesterone-binding B subunit, and monoclonal BF4 against the non-progesterone-binding 90,000-mol-wt protein component. Chickens were immature animals with or without estrogen priming, and with or without progesterone treatment. The antibodies were revealed by means of an immunoperoxidase technique that used the avidin-biotin-peroxidase complex, and controls were performed by presaturation of antibodies with the purified 8S-progesterone receptor, the B subunit, and 90,000-mol-wt protein.

The progesterone receptor was detected not only in well-characterized target tissues, i.e., in glands and luminal epithelium, but also in stromal cells (some displayed the strongest reaction), in mesothelium, and in fibers of smooth muscles. Only in cell nuclei, whether or not the animals received an injection of progesterone was an antigen revealed corresponding to the B subunit (and/or to the A subunit, because there is immunoreactivity of IgG-RB with both hormone-binding subunits A and B). The 90,000-mol-wt protein was revealed in both cytoplasm and nuclei. These immunohistological data suggest that the concept of steroid action that necessarily involves the original formation of the hormone-receptor complexes in the cytoplasm before translocation to the nucleus, may have to be revised.

In biochemical and histological studies, steroid hormone receptors have usually been studied using radioactive hormonal ligand binding. Only recently, antibodies to steroid receptors have become available (see the review in reference 1), allowing the detection of the receptor molecules themselves, independently of hormone binding. Therefore it becomes possible to reveal histologically both steroid-free and -occupied receptors under several physiological, pathological, and pharmacological conditions, and in particular, to compare the receptor state in untreated and steroid hormone-treated animals.

In spite of the increasing availability of antibodies to receptors, there are only a few published immunohistochemical

studies. The glucocorticosteroid receptor has been demonstrated in cultured cells, using immunofluorescence (2, 3). The estrogen receptor has been observed in rat pituitary gonadotropic, lactotropic, and somatotropic cells at the electron microscopic level (4), and in tumoral mammary cells at the light microscope level (5). The difficulties encountered in this new way of studying steroid hormone receptors may come from the lack of monospecificity of polyclonal antibodies raised against highly, but not completely, purified receptor. With monoclonal antibodies, a recent report reveals the estrogen receptor in a number of estrogen target cells of mammalian reproductive tissues (6). In any case, the fixation condi-

tions are critical, in that they should not provoke the displacement of the receptor within the cell or alter the antigenic determinants.

The chick oviduct is a system of choice for the study of steroid hormone action. In particular, progesterone is involved in differentiation and protein synthesis of oviduct cells in the glands and the luminal epithelium (7, 8). The progesterone receptor (PR)<sup>1</sup> is increased after estradiol treatment of the chicken (9). We have obtained polyclonal antibodies IgG-G3 (10), raised against the nontransformed "8S" form of PR (8S-PR). The 8S-PR preparations contain the A and B progesterone-binding subunits ( $M_r = 79,000$  and  $110,000$ , respectively; see reference 11) and a nonprogesterone-binding protein ( $M_r = 90,000$ ; see references 12 and 13); the tentative stoichiometry is one molecule of A or B for two molecules of  $90,000 M_r$  per one 8S-PR oligomere (14). IgG-G3 interact with both the progesterone-binding subunits B and A and with the nonprogesterone-binding component ( $90,000 M_r$  protein) (12). We also raised polyclonal antibodies IgG-RB (15) against the purified B subunit (16), which react with both B and A but not with the  $90,000 M_r$  protein. Furthermore, we have described a monoclonal antibody BF4 (17) that recognizes the  $90,000 M_r$  protein but not A and B. The present report gives a full account of the observations made with the three antibodies and that use improved conditions of fixation. Progress towards understanding of the structure of the chick oviduct PR (12–14) helps to interpret the immunohistochemical observations. Preliminary publications have reported a few data obtained with IgG-G3 and BF4 (18), and with IgG-RB (19).

## MATERIALS AND METHODS

**Antibodies:** Antibodies have been recently described in references 10, 15, and 17. The specificity studies included density-gradient experiments and Western immunoblotting analysis on crude and purified receptor components.

IgG-G3 (10) is an immunoglobulin fraction of an antiserum raised in a goat against the nontransformed, molybdate-stabilized 8S-PR (20). It recognized the nontransformed 8S-PR, as well as its constituents released after transformation of the receptor, the subunits A and B and the  $90,000 M_r$  component (12). IgG-RB (15) is the immunoglobulin fraction of an antiserum raised in a rabbit against the purified B subunit of PR (16). IgG-RB reacted with B and A subunits (15). BF4 (17) is a rat monoclonal antibody obtained against the nontransformed 8S-PR, and interacting selectively with an immunogenic determinant of the  $90,000 M_r$  component (13). This  $90,000 M_r$  protein was shown to be present also in the untransformed, molybdate-stabilized 8S-forms of other steroid hormone receptors, for androgens, estrogens, and glucocorticosteroids (13), and it is present in other chick cells in equal or even higher amount than in the oviduct cell, (Catelli, M. G., N. Binart, B. Moncharmont, and J.-M. Gasc; unpublished observations).

The polyclonal antibodies were routinely used after presaturation with, either for IgG-G3, ovalbumin, conalbumin ( $50 \mu\text{g/ml}$ ), and normal chicken serum (1:100), or, for IgG-RB, normal chicken serum alone (1:100).

**Preparation of Animals and Tissues:** Chickens of the Warren strain were used. 1-wk-old female chickens received, by intramuscular injection, 1 mg of estradiol benzoate in sesame oil for 10 d. After an interruption of 1 wk, they again were injected with five daily identical injections. At the end of the second stimulatory treatment, the oviduct weighed  $\sim 1.8$  g and cells contained a mean of  $\sim 40,000$  [<sup>3</sup>H]progesterone binding sites per cell (21). In some experiments, oviducts were used before any hormonal treatment of chicken

(immature animals), or after only five estradiol injections (primostimulation), or after a withdrawal of 4 wk following the last injection of the 10-d initial estradiol treatment. After decapitation the oviduct was immediately removed. A slice was taken in the middle part of the magnum, washed in cold PBS, cut into two to four pieces, and fixed in appropriate fixative fluids. The longest side of each piece was not  $> 5$  mm.

Several fixatives were used and compared. Fixation, washes, and dehydration processes were carried out in an ice bath. The best detection of PR was obtained with the four following fixatives: (a) Bouin's fluid for 60–80 min, followed by several washes in 70% ethanol; (b) Carnoy's fluid for 60–80 min, followed by several washes in absolute ethanol; (c) glutaraldehyde (0.5%) in 0.1 M Soerensen buffer, pH 7.4, for 90 min, followed by several washes in either Soerensen buffer or PBS; (d) absolute ethanol + 1% acetic acid for 60–80 min, followed by several washes in absolute ethanol.

Dehydration was made in graded ethanol when needed, and terminated in 1-butanol before embedding in paraffin. The whole process from fixation to embedding was carried out within 7 h.

**Immunohistochemical Procedure:** Paraffin sections (thickness  $7 \mu\text{m}$ ) were deparaffinized, rehydrated, and rinsed in PBS. The immunoperoxidase technique included the use of a biotinylated secondary antibody and avidin–biotin–peroxidase complex to label the secondary antibody (Vectastain reagents, Vector Laboratories, Burlingame, CA). Sections were first incubated in 3% nonimmune serum of the animal species in which the biotinylated secondary antibody was raised. This and all subsequent steps were carried out at room temperature. The excess of serum was removed and the primary antibody was placed on the sections (90–120 min). After washing in PBS, sections received the secondary biotinylated antibody (dilution 1:400; 30 min). The secondary antibody was washed in PBS and replaced by the avidin–biotin–peroxidase complex (30 min). After washing, the peroxidase activity was revealed by 3-3'-diaminobenzidine tetrahydrochloride (0.5 mg/ml) in presence of 0.01%  $\text{H}_2\text{O}_2$  in Tris buffer, pH 7.6. Sections were then rinsed, dehydrated, and mounted.

The total protein concentrations of the three antibody preparations and their usual dilutions were 40 mg/ml and 1:1,000 to 1:4,000 for IgG-G3, 13 mg/ml and 1:250 to 1:500 for IgG-RB, and 2 mg/ml and 1:1,000 to 1:4,000 for BF4.

Controls were run on adjacent sections placed on the same histological slide. They included immunoglobulin fractions from nonimmune or preimmune sera, dilution of specific antibodies up to extinction of staining, and presaturation with heterologous antigens (ovalbumin, conalbumin, calmodulin, avidin) and with different preparations of PR. The 8S-PR used for presaturation was obtained after affinity chromatography and ion-exchange (DEAE-Sephacel, Pharmacia, Uppsala, Sweden) chromatography in presence of  $\text{Na}_2\text{MoO}_4$ ; this purified 8S-PR preparation was used after concentration on DEAE-Sephacel in absence of molybdate, a maneuver yielding activated receptor preparation containing A, B, and  $90,000 M_r$  protein (12). The B subunit was prepared as described (16). The  $90,000 M_r$  protein was prepared by immunoaffinity chromatography on Sepharose-BF4 (Catelli, M. G., and B. Moncharmont, unpublished method). For presaturation, antibody (see dilutions above) and antigen ( $2\text{--}10 \mu\text{g/ml}$  of PR components and  $> 10 \mu\text{g/ml}$  for other antigens) were mixed at the desired dilution and incubated overnight at  $4^\circ\text{C}$ . Mixtures were then used on sections instead of antibodies alone. For BF4, the nonimmune control was obtained with culture medium of an IgG-nonsecreting myeloma line added to nonimmune rat IgGs at the same concentration as in BF4 preparations.

All controls not including an anti-PR antibody were negative, except for the endogenous peroxidase activity of red blood cells. However, a slight positivity of goat nonimmune IgGs was observed in some cells of the luminal epithelium after glutaraldehyde fixation.

## RESULTS

For each of the three antibodies, we describe the most representative data obtained in the oviduct of chicken having received the second estrogen stimulation, using the four fixation procedures. Thereafter we report on animals under other hormonal treatments.

### Immunohistochemical Studies with IgG-G3

When treated with IgG-G3, sections of oviduct from estradiol-injected chickens showed specific staining in cytoplasm and nuclei of cells of the luminal epithelium, glands, stroma, and mesothelium. The nuclei of smooth muscle fibers were also positive. The most complete staining was observed after glutaraldehyde (Fig. 1a) and acidified-ethanol fixations. The histological quality however was often poor, and staining in

<sup>1</sup>Abbreviations used in this paper: BF-4, rat monoclonal antibody obtained against the nontransformed 8S progesterone receptor; IgG-G3, immunoglobulin fraction of antiserum raised in a goat against the nontransformed 8S progesterone receptor; IgG-RB, immunoglobulin fraction of an antiserum raised in a goat against the B subunit of progesterone receptor; PR; progesterone receptor; 8S-PR, nontransformed 8S progesterone receptor.

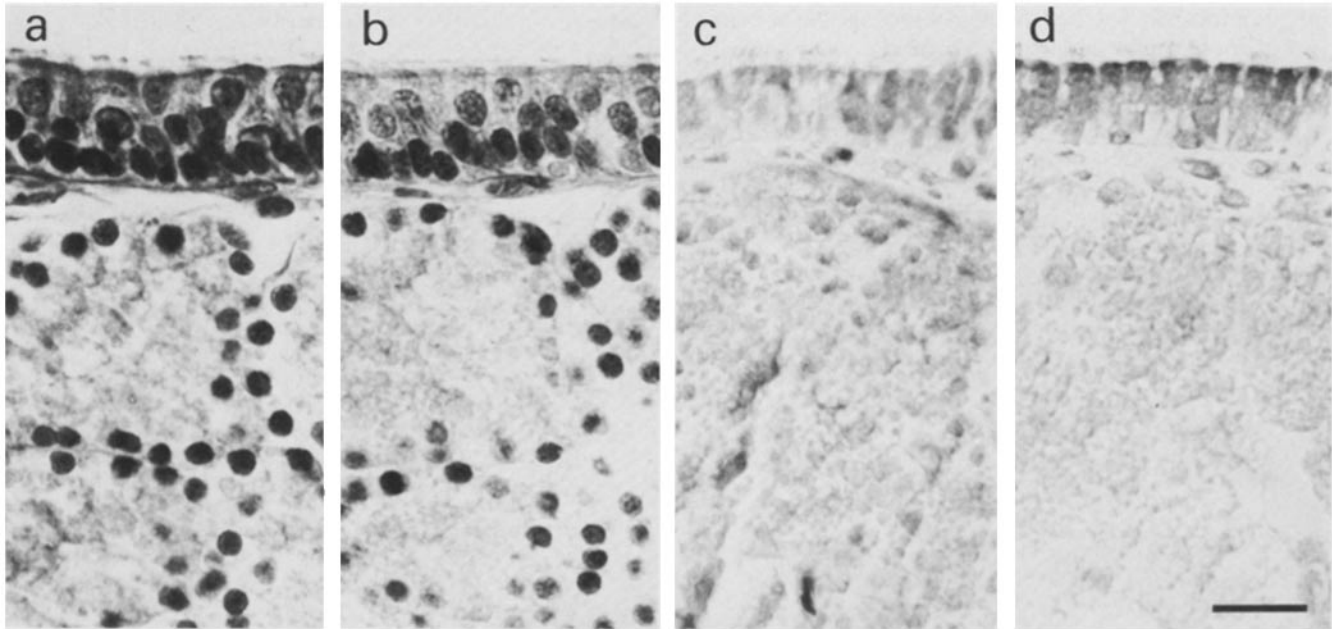


FIGURE 1 Immunoperoxidase staining with IgG-G3 of estradiol-treated chick oviduct sections, in absence of progesterone treatment. A strong reaction is observed in cytoplasm and nuclei of the luminal epithelium and in nuclei of glands and stroma (a). The cytoplasm of glandular and stromal cells is only weakly stained. When IgG-G3 was presaturated by purified 90,000  $M_r$ -protein (b), the cytoplasmic staining in all cell types was reduced up to near extinction, whereas the nuclear staining is only weakly reduced. When IgG-G3 was presaturated simultaneously by the purified 90,000- $M_r$  protein and B subunit (c) all staining, cytoplasmic and nuclear, was abolished. Preimmune immunoglobulins (d) produce in the luminal epithelium the weak, but constant, staining that appears to be similar in intensity to the weak staining observed after presaturation by 90,000- $M_r$  protein (b) or 90,000- $M_r$  protein and B subunit (c). Glutaraldehyde fixation; no counterstaining. Bar, 20  $\mu\text{m}$ .  $\times 650$ .

the cytoplasm of the luminal epithelium and glands were weak. After Carnoy's fixation there was no nuclear staining, and after Bouin's fixation the glands were only weakly and occasionally stained.

After presaturation of IgG-G3 with 8S-PR preparation, all staining was abolished in all cell types of the oviduct, both in cytoplasm and nuclei (not shown). Presaturation of IgG-G3 with the 90,000- $M_r$  protein abolished the cytoplasmic staining (incompletely, however, after glutaraldehyde fixation), whereas the nuclear reaction was apparently not, or only slightly modified (Fig. 1 b). The remaining weak but constant cytoplasmic staining observed with glutaraldehyde fixation did not disappear even after a large excess of 90,000- $M_r$  protein; it should be noted that similar weak reaction was always obtained in the cytoplasm of epithelial cells treated with nonimmune goat serum and fixed with glutaraldehyde (Fig. 1 d). Presaturation of IgG-G3 simultaneously with the 90,000- $M_r$  protein and B subunit suppressed the cytoplasmic and nuclear staining (Fig. 1 c), except for a weak cytoplasmic staining in the luminal epithelium, similar to that reaction observed with preimmune IgGs (Fig. 1 d).

#### Immunohistochemical Studies with IgG-RB

With IgG-RB, a nuclear staining was observed in the cells of the luminal epithelium, glands, stroma (Fig. 2 a), mesothelium (Fig. 2 c), and smooth muscular cells (Fig. 2 d), after all fixation procedures but the Carnoy's. This negative result confirmed that the Carnoy's fixative is unable to preserve, on sections from the same oviduct, the nuclear antigens revealed with the three other fixations. No cytoplasmic staining competitive by any PR preparation was observed in luminal epithelial and glandular cells.

Presaturation of IgG-RB with 8S-PR (not shown) as well as with the B subunit (Fig. 2 b) abolished all nuclear staining in all cell types. Conversely presaturation with a purified 90,000- $M_r$  protein did not modify the nuclear staining in any type of cells (not shown).

#### Immunohistochemical Studies with BF4

With all fixatives, an intense staining was observed in cytoplasm of epithelial cells after treatment with the BF4 monoclonal antibody and a positive reaction also was observed in nuclei, although less strongly (Fig. 3 a). Nuclei were also well stained in glandular cells (Fig. 3 a), stroma, mesothelium (Fig. 3 e), and muscular cells. However, only acidified alcohol fixation yielded a strong and constant reaction in the cytoplasm of glandular cells (Fig. 3 b) and of other cell types.

Presaturation by the 8S-PR (not shown) or the 90,000- $M_r$  purified protein (Fig. 3 c) resulted in complete extinction of all reactions in nuclei and cytoplasm. Presaturation with the B subunit did not reduce the staining in nuclei and cytoplasm (Fig. 3 d).

As with the two other antibodies, the most complete distribution of staining was observed after acidified ethanol and glutaraldehyde fixations, which, however, frequently gave a poor histological picture (Fig. 3 a).

In Fig. 3 e, we show immunostaining of mesothelium cells with BF4: both cytoplasm and nuclei are positive, in contrast to the only nuclear localization of antigen revealed by IgG-RB (Fig. 2 c).

#### Animals under Different Hormonal Conditions

Chickens during primostimulation by estrogen showed the

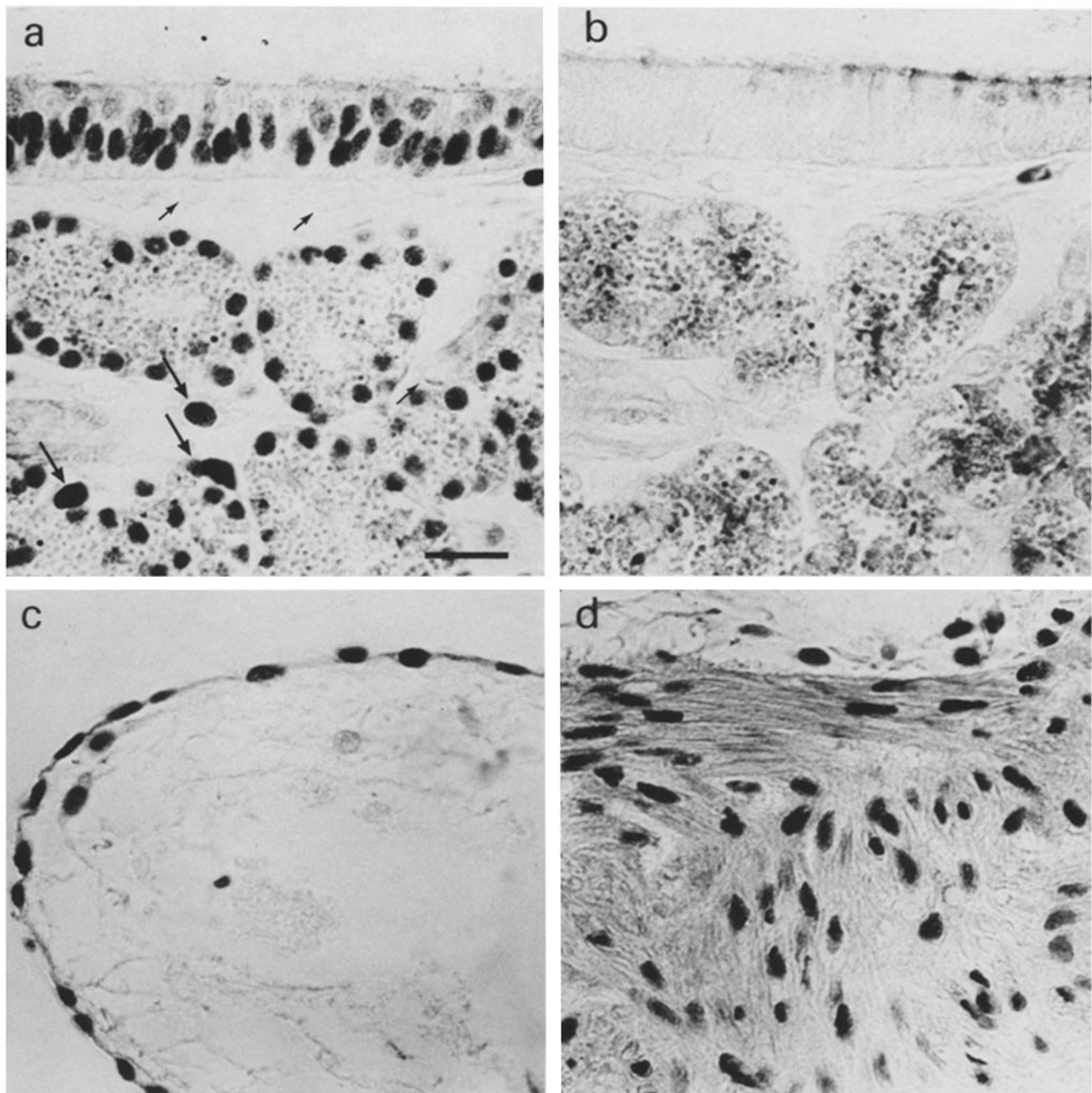


FIGURE 2 Immunoperoxidase staining with IgG-RB of estradiol-treated chick oviduct sections, in absence of progesterone treatment. Cell nuclei in the luminal epithelium, the glands, and the stroma are positive when the antibody is applied alone (a), whereas no nuclear staining remains when the antibody was presaturated by a purified preparation of the B subunit of PR (b). Some stromal cells (long arrows in a) are very strongly stained, and others (short arrows in a) are not stained. In the luminal epithelium two groups of nuclei differ by the intensity of the reaction: those located at the basis of the epithelium are as strongly stained as glandular cell nuclei, whereas those at the apex of the epithelium are weakly or not stained. (c) The estradiol treatment induces on the outer surface of the oviduct, between the smooth muscle fibers and the mesothelium, the formation of vesicles full of fluid and loose stromal tissue; such a vesicle is shown here bordered by mesothelial cells whose nuclei are strongly stained by the reaction with the anti-B subunit antibody. (d) In smooth muscle fibers, a reaction with the anti-PR antibody is observed in the nuclei. The weak cytoplasmic reaction is due to a contaminant as indicated by the lack of saturability of this reaction by a purified B subunit preparation which completely abolishes the nuclear staining (not shown). The presence of nuclear PR revealed by IgG-RB is remarkable because no progesterone was administered to the animal. (a, b, and d) Glutaraldehyde fixation. (c) Bouin's fixative; no counterstaining. Bar, 20  $\mu$ m.  $\times$  650.

same distribution of positive cells with the three antibodies as at the end of the secondary stimulation.

In animals withdrawn from estrogen treatment for 4 wk, the staining with IgG-G3 was weak and mostly nuclear (not shown). With IgG-RB, it was only nuclear (Fig. 4a). BF4 only

reacted in the cytoplasm of luminal epithelium and glandular cells (Fig. 4b). The BF4 staining in the cytoplasm of the glands was more easily revealed in withdrawn animals than in fully stimulated chickens, possibly because the absence of secretory granules allowed a better reaction of the receptor

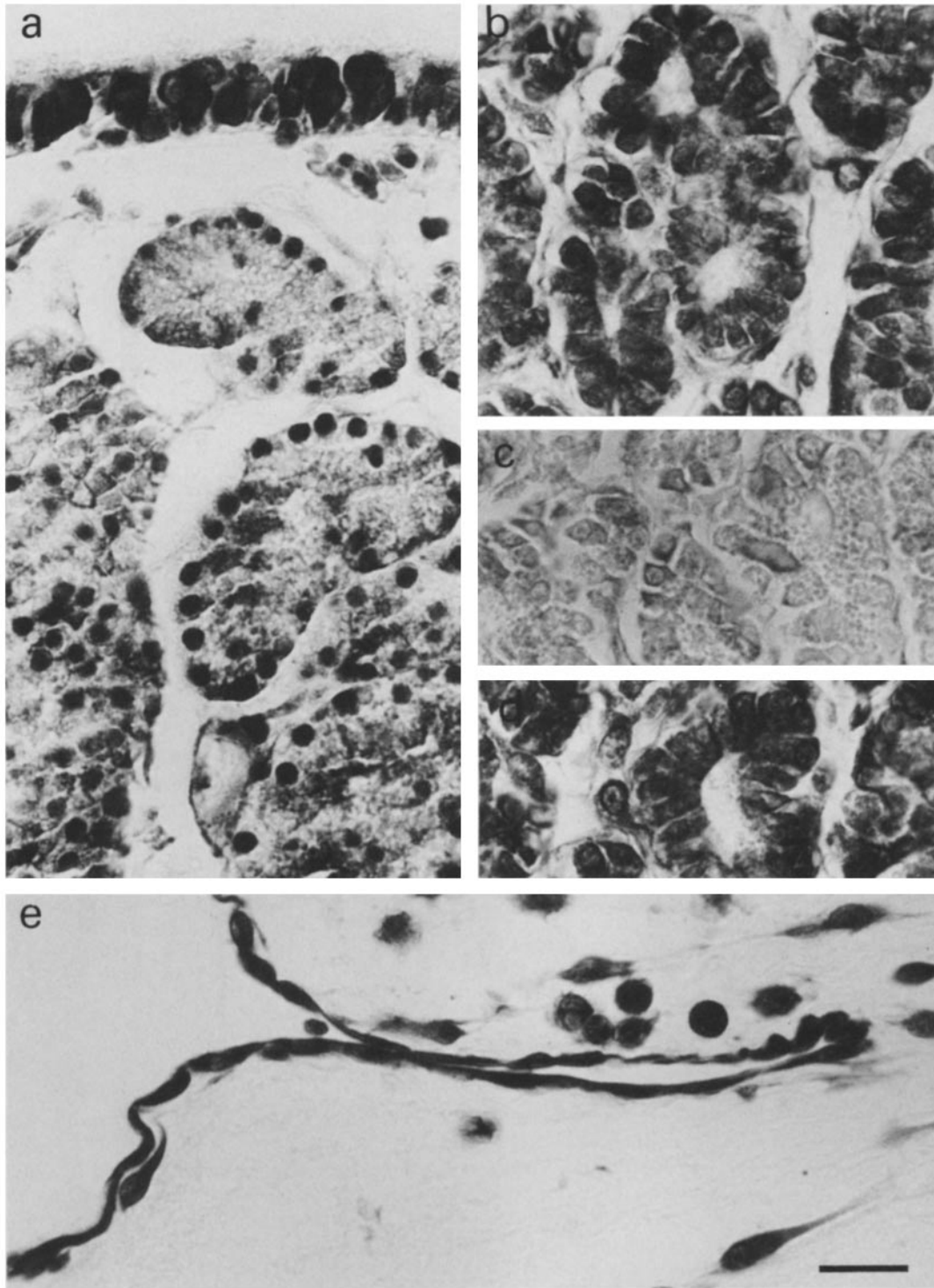
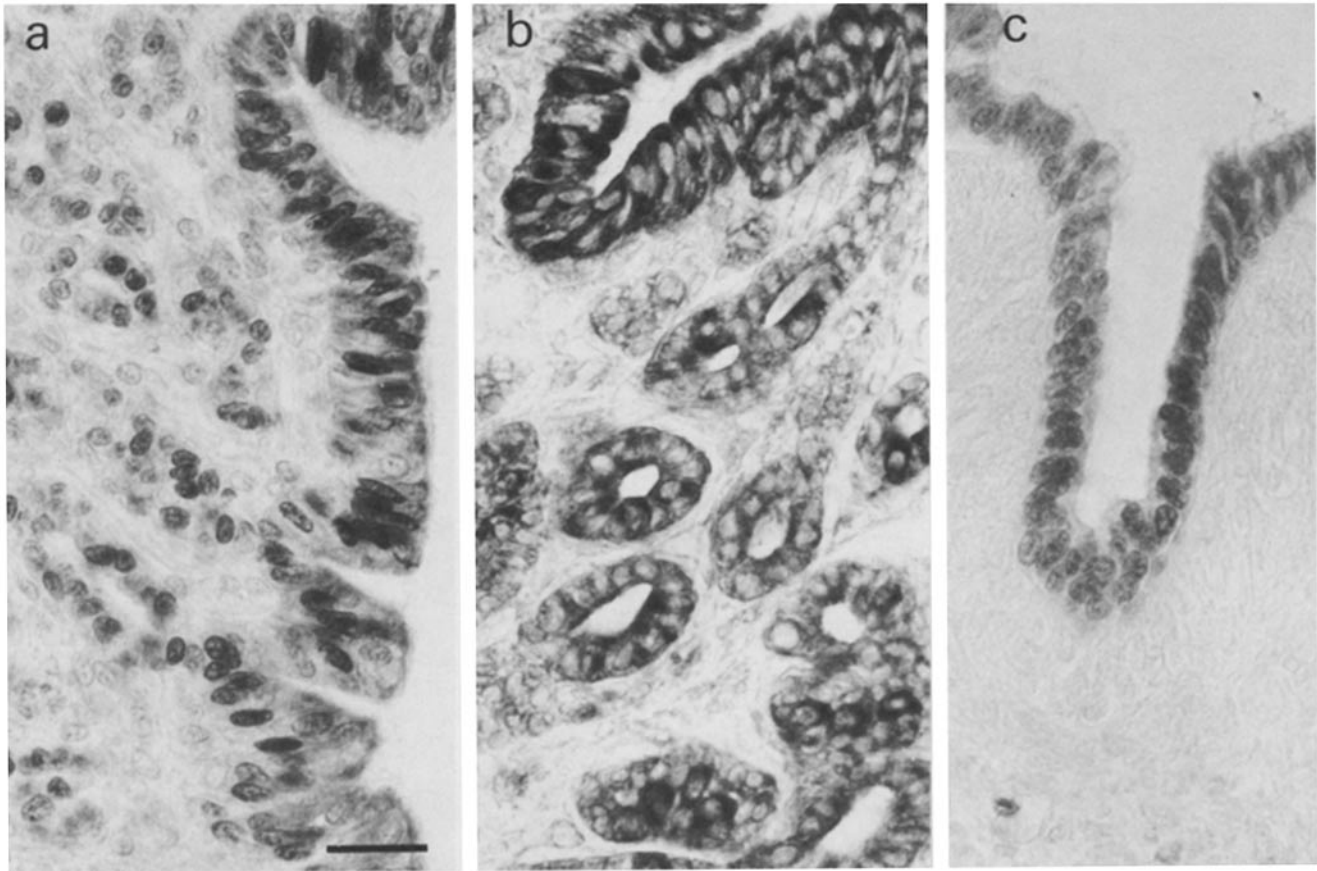


FIGURE 3 Immunoperoxidase staining with BF4 of estradiol-stimulated chick oviduct sections, in absence of progesterone treatment. After glutaraldehyde fixation (a), BF4 reveals the antigenic determinant of the 90,000- $M_r$  protein in cytoplasm and nuclei of the luminal epithelium, and in nuclei of glands and stroma. In cytoplasm of glands the reaction is weak. The poor histological quality of the section is the consequence of an insufficient fixation in glutaraldehyde necessary for the preservation of the antigenic determinant in nuclei. After acidified-alcohol fixation (b), BF4 reacts in cytoplasm and nuclei of the glands with equal intensity. All staining is abolished (c) when BF4 was preincubated with purified 90,000- $M_r$  protein, whereas the staining is unchanged (d) after preincubation with the B subunit. In mesothelial cells (e) BF4 stains cytoplasm and nuclei, unlike IgG-RB which in the same cells reveals the B subunit only in the nuclei (Fig. 2c). (a) Glutaraldehyde fixation; (b, c, d, and e) Acidified alcohol fixation; no counterstaining. Bar, 20  $\mu\text{m}$ .  $\times 650$ .

with the antibody. Glutaraldehyde, which best permitted the detection of the 90,000- $M_r$  protein in estrogen-stimulated animals, was not used with oviduct of withdrawn chickens.

For that reason we cannot rule out the presence of 90,000- $M_r$  protein in nuclei of oviducts of the withdrawn chickens.

Immature chickens that never received any estradiol injec-



**FIGURE 4** Immunoperoxidase staining of PR of chick oviduct sections, in absence of estradiol and progesterone treatment. (a) Immunoperoxidase staining with IgG-RB of a chick oviduct section, after 4 wk of hormonal withdrawal. The B subunit is revealed in nuclei of glands and luminal epithelium. The presence of a weak cytoplasmic reaction in the epithelium cannot be excluded. However, the nuclear staining appears much stronger although this animal never received progesterone, and had not received estradiol for 4 wk. (b) In the same animal as in a, the immunoperoxidase staining with BF4 is still strong in the cytoplasm of the luminal epithelium and weak in nuclei. In the glands, almost devoid of secretion granules, the 90,000-*M<sub>r</sub>* protein is more easily revealed than in estradiol-stimulated oviduct (see Fig. 3 a). (c) Immunoperoxidase staining with IgG-RB of an oviduct section from immature, untreated chicken. As in withdrawn animals (a) the cytoplasm may also be weakly stained. The size and density of the nuclei in the epithelium leaves little space to observe clearly the cytoplasm and no definitive conclusion can be drawn as to the presence of small amounts of cytoplasmic B subunit. (a and c) Bouin's fixative; (b) Carnoy's fixative; no counterstaining. Bar, 20  $\mu\text{m}$ .  $\times 650$ .

tion had no glands. The oviduct was only composed of a stroma lined inwards with the luminal epithelium and outwards with some muscle fibers and a flat epithelium (mesothelium). Cells of the luminal epithelium were stained with IgG-G3 in the cytoplasm and nuclei, with IgG-RB in nuclei only (Fig. 4c), and with BF4 in the cytoplasm and also weakly in the nuclei. Cells of the mesothelium were occasionally stained in nuclei with IgG-RB. The stromal cells showed a weak and diffuse reaction with BF4 that could not be distinguished with certainty from a background staining.

In all these different hormonal conditions, presaturation experiments always gave identical results to those obtained at the end of the secondary estrogen stimulation.

In addition, studies were also conducted in progesterone- or dexamethasone-treated chickens, either fully stimulated by the second estrogenic stimulation, after the primary stimulation, or in the withdrawn state; they were killed 1 h after hormone injection. The same distribution of positive cells was observed as in Fig. 1-4 for animals not receiving these two hormones. In particular, the distribution of positive cells,

and the relative intensity of staining in the cytoplasm and the nuclei were the same.

Semiquantitative estimations were carried out with IgG-G3 and IgG-RB. The intensity of the nuclear staining was related to the extent of estradiol stimulation: it was greatest after the secondary estrogen stimulation, followed by the primary stimulation, then the withdrawn state, and finally the nonstimulated animals.

#### DISCUSSION

##### *PR Subunits Are Revealed in Target Cells with Specific Antibodies and Suitable Fixation Conditions*

The immunohistochemical data reported here should be interpreted in the light of the properties of the antibodies used. IgG-G3 recognizes the intact nontransformed 8S-PR, as well as its progesterone-binding components A and B, and nonprogesterone-binding 90,000-*M<sub>r</sub>* protein (12), although the definitive stoichiometry in the 8S-PR preparation is not

known (14). One should take also into account that the nontransformed "8S" forms of other steroid hormone receptors also contain the 90,000- $M_r$  protein antigen (13). IgG-RB (15) does not detect the 90,000- $M_r$  protein, but reacts with both B and A subunits of the progesterone receptor. Finally, only BF4 reveals the 90,000- $M_r$  protein; we have already seen that this is a component of all tested 8S-steroid receptors and that there is an excess of 90,000- $M_r$  protein over the amount necessary to account for these receptors; the reason for this is unknown.

The techniques presented here have been worked out after many different trials to optimize the fixation conditions and to best preserve the antigenic determinants. A suitable fixation of glands is difficult mostly because of the large abundance of secretory granules, which is an obstacle to the reaction of other antigens with their antibodies (for an example, compare Figs. 3a and 4b). Antigenic preservation is satisfactory only when the tissues are left in the fixatives for 60–90 min. Previously we had used 5–6-h fixation times, but it appeared that small pieces of tissue were usually overfixed in such conditions. Glutaraldehyde had been used at 1%, instead of 0.5% now. These modifications account for the differences between our present and preliminary observations (18). Comparative studies with frozen cryostat sections are currently underway; our first results (19) are in full agreement with those obtained here with paraffin sections.

This work reveals, besides the expected PR-containing target cells in glands and the luminal epithelium, other target cells in stroma, smooth muscles, and mesothelium. The rather strong reaction recorded in some stromal cells (Fig. 2a) should lead to more research to discover the effects of progesterone in these cells.

### *PR Subunits Are All Revealed by Specific Antibodies in Target Cell Nuclei*

The use of the three antibodies, and the availability of three related antigens in purified forms that were used in presaturation experiments, allowed precise examination of the subcellular distribution of the PR components. In the cytoplasm, there is no immunoreaction with IgG-RB, whereas the immunoreaction observed with IgG-G3 and BF4 is completely abolished by presaturation with 90,000- $M_r$  protein (and naturally also 8S-PR). We conclude that the 90,000- $M_r$  protein is present in the cytoplasm, and that no antigen corresponding to the B or A subunit is detectable under our conditions, whatever the hormonal condition is. In the nuclei, the three antibodies give an immunoreaction, but the results of the presaturation experiments differ according to the case: IgG-RB staining is completely extinguished by purified B and by 8S-PR, but not by 90,000- $M_r$  protein; the staining by BF4 is abolished by the 90,000- $M_r$  protein and 8S-PR, but not by the purified B; the reaction observed with IgG-G3 is abolished by 8S-PR or by the simultaneous addition of B and 90,000- $M_r$  protein; however, extinction is only partial after presaturation with only the B subunit, the remaining staining being attributable to either some 90,000- $M_r$  protein or to residual staining of the cytoplasm surrounding and underlying the nucleus. The decrease of IgG-G3 nuclear staining by presaturation of the 90,000- $M_r$  protein alone is difficult to assess, and weak in any case. We conclude from these data that there exist in nuclei both progesterone-binding subunits A and/or B and 90,000- $M_r$  protein.

### *Histochemical Nuclear Receptor vs. Biochemical Cytosol Receptor*

In terms of receptor subcellular distribution, there is much contradiction between the immunohistochemical data and the biochemical results published so far. Measurements of specific progesterone-binding macromolecules in estrogen-stimulated animals, in absence of progesterone, indicate that they are mostly, if not totally, found in the cytosol (high-speed supernatant) fraction of the homogenate, and conversely absent in the nuclear fraction (21). This seems in strict opposition to what is observed by immunohistochemistry, which only demonstrates the presence in nuclei of the progesterone-binding receptor units A and B. It is very unlikely that the immunohistochemical observations are due to artifactual translocation of PR from cytoplasm to nucleus during fixation. Recently, the nuclear localization of PR was confirmed on frozen sections (19), i.e., is under conditions excluding almost certainly such an artifact. Cryostat sections were obtained from chick oviduct explants and incubated *in vitro* in presence of the radioactive progestin [ $^3\text{H}$ ]Org 2058. They were processed for autoradiography and/or immunohistochemistry with IgG-RB. At the periphery of the explants, cell nuclei were revealed by both techniques, indicating the nuclear localization of hormone-PR complexes. In cells in the central part of the explants, no radioactive ligand was detected, indicating that the hormone had not reached the inner cells, whereas PR was still detected immunohistochemically in the nuclei. No cytoplasmic receptor was observed. Therefore, one now should envisage that the biochemical results obtained after homogenization may be due to the artificial relocation of PR in the cytosol fraction of the homogenate. Uncontrolled extraction from the nucleus during the homogenization process would be due to the low affinity of PR for nuclear constituents, since it is nontransformed in absence of hormone (review in reference 22). However, this hypothesis has yet to be directly substantiated. The absence of immunostaining with IgG-RB in the cytoplasm may be even a false negative result, the corresponding antigen being altered during the procedure and/or present in too low levels to be detected. It is also possible that low levels of endogenous progesterone in immature chickens are sufficient to promote the location of the receptor in the nucleus, but not the hormonal response which would require higher levels of progesterone. In any case, immunohistological evidence has been presented for the presence of steroid receptor in the cytoplasm of several target cells (2–5).

### *Nuclear "Inactive" Receptor and Hormone Action*

Measuring the receptor in the nuclear fraction of oviduct homogenate after hormonal exposure has been interpreted in terms of "translocation" into the nucleus, based on its presence in the cytosol in absence of hormone. The immunohistological observation of the nuclear location of PR, whether or not progesterone has been administered, argues against the concept that the initial formation and location of hormone-receptor complexes in the cytoplasm is mandatory to trigger steroid hormone action in the target cells. Therefore the results reported in this paper challenge the dogma designating the transfer to the nucleus as a limiting step in the cellular response. If all or some of the receptor is already situated in the nucleus in absence of hormone, then the hormone may reach it directly there, and eventually provokes its "transfor-

mation" or "activation" in situ, this last step being possibly decisive in the response to hormone. Transformation or activation is poorly understood: it is usually defined by the increase of affinity for a nuclear structure called "acceptor" on the basis of binding to nuclei, and this fits well with the recovery of hormone-receptor complexes in the nuclear fraction of homogenates. The recovery in the nuclear fraction of all hormone-binding components of the receptor, but not of nontransformed 8S-receptor, would then result from the increased affinity of the hormone-receptor complexes after dissociation of the oligomere, releasing the 90,000- $M_r$  protein from hormone-binding units, which probably would be accompanied by changes of conformation of these subunits. That the presence of receptor in the nuclear fraction is not necessarily linked to agonist hormonal activity, has been already suggested when it was observed that such antihormones as antiestrogen (23) or antiglucocorticosteroid (24), even though displaying no agonistic hormonal activity, also increase the binding of the receptor for the nuclear fraction. It has already been shown that the estradiol receptor devoid of hormone is almost all nuclear in the liver of immature female and male chickens (25). Moreover, when the present work was complete and the manuscript ready to be sent out, two interesting communications, dealing with the mammalian estrogen receptors, confirmed our recent (19) and present findings with the chick PR: both the results obtained with monoclonal antibodies to estrogen receptor (6) and the data observed after separation of nuclei by cytochalasin B (26) also indicated the nuclear localization of the cytosol (nonoccupied) and nuclear forms of the receptors.

We then have to admit that the presence of the receptor in the nucleus, whether unfilled by hormone or complexed to antihormone, does not lead per se to hormone action. Therefore, one should distinguish "really active" hormone-receptor complexes, which naturally must be nuclear in order to promote hormone action, from nonactive receptor (free or binding antihormone), which may also be situated in the nucleus. It is therefore not "translocation" from cytoplasm to nucleus that then definitively differentiates receptors engaged in hormone action from receptors not engaged in hormone action. What is important is the nature of interaction(s) of the active form of the receptor with the chromatin machinery. Recent biochemical studies have indicated the selective binding in chromatin of (active) hormone-receptor complexes, but not of antihormone-receptor complexes (27). The initial (in absence of hormone) distribution of the receptor between the nucleus and the extra nuclear compartment of the cell may vary according to species, organs, and hormones, and not be crucial for determining the hormone response.

In summary, this report on immunocytochemical localization of PR in the chicken oviduct indicates, owing to the use of different fixation procedures and to availability of several antibodies and preparations of PR components (a) that PR is present in most cell types of the differentiated oviduct of estrogen-treated chickens, but only in the epithelial cells of the immature, non-estrogen-treated chicken oviduct; (b) that the PR is present in the cell nuclei of immature chickens, and that progesterone administration does not modify significantly the picture; (c) that there is no direct or indirect evidence for the presence of the progesterone-binding B and/or A subunits of PR in the cytoplasm of estrogen-stimulated chicken oviduct; (d) that the 90,000 non-progesterone-bind-

ing protein component of nontransformed 8S-PR is present in the cytoplasm and the nuclei of the same cells containing the B and/or A subunits in their nuclei; (e) that the concept of steroid action involving necessarily the formation in the cytoplasm of hormone (antihormone)-receptor complexes may have to be revised. In fact, with respect to this last point, we suggest that the necessary and sufficient active hormone-receptor complexes may be formed in the nucleus in which the unoccupied receptor is originally present and where the gene response will take place.

We thank Francine Delahaye for her skilled technical assistance, Maria-Grazia Catelli and Bruno Moncharmont for the gift of purified 90,000- $M_r$  protein, Thierry Bouchou for the gift of purified B subunit, and Françoise Boussac, Martine Rossillon, and Jean-Claude Lambert for assistance in the preparation of the manuscript.

Received for publication 30 March 1984, and in revised form 4 June 1984.

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