Van Deenen, L. L. M., and DeGier, J. (1974), in The Red Blood Cell, Surgenor, D. MacN., Ed., New York, N.Y., Academic Press, p 147.
Walter, H. (1975), *Methods Cell Biol. 9*, 25.
Walter, H., and Coyle, R. P. (1968), *Biochim. Biophys. Acta 165*, 540.
Walter, H., Edgell, M. H., and Hutchison, C. A., III (1970), *Biochim. Biophys. Acta 204*, 248.
Walter, H., Krob, E. J., Ascher, G. S., and Seaman, G. V. F. (1973), *Exp. Cell Res. 82*, 15.
Walter, H., Miller, A., Krob, E. J., and Ascher, G. S. (1971), *Exp. Cell Res. 69*, 416.
Walter, H., Selby, F. W., and Garza, R. (1967), *Biochim. Biophys. Acta 136*, 148.
Walter, H., Tung, R., Jackson, L. J., and Seaman, G. V. F. (1972), *Biochem. Biophys. Res. Commun. 48*, 565.

# Inhibition of Estrogen-Receptor–DNA Interaction by Intercalating Drugs†

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ABSTRACT: Ethidium bromide, an intercalating drug, was shown to inhibit the in vitro DNA binding of the uterine estradiol-receptor complex. The inhibition was reversible, dose dependent, complete for total saturation of DNA intercalating sites by the dye, and proportional to the extent of intercalated drug. The binding of the receptor to phosphocellulose and poly(adenylic acid)-cellulose was not decreased by this drug. Similar inhibition was also obtained with 9-hydroxyellipticine. Denatured DNA was more efficient at binding the estrogen receptor than phosphocellulose or poly(adenylic acid)-cellulose but less efficient than native DNA. We conclude that the DNA binding of the estrogen receptor cannot be simply interpreted in terms of electrostatic interactions but requires a particular double-helical structure of DNA.

In the target cell estradiol translocates its cytosol receptor protein in the nucleus where the resulting complex interacts with nuclear acceptor(s) (Jensen and De Sombre, 1973). It has been postulated that DNA may represent at least a part of such an acceptor, since the binding of estradiol–receptor complexes to DNA has been demonstrated in vitro (Toft, 1973). However, the biological significance of such an interaction remains unclear, since no sequence DNA specificity has been proved. Ethidium bromide and 9-hydroxyellipticine (Figure 1) are cationic dyes that at low concentrations modify the three-dimensional structure of DNA by intercalating between base pairs; at higher concentrations, they bind weakly to phosphate groups of DNA (Waring, 1965; Le Pecq and Paoletti, 1967; Le Pecq et al., 1974). In the present study, we investigated the binding of the estrogen receptor to DNA after modifying its helical structure with intercalating drugs.

## Experimental Procedure

*Materials.* [$^3$H]Estradiol, 60 Ci/mmol ($^3HE_2$), was obtained from CEA (Saclay, France), ethidium bromide from Boots Pure Drug Co. (Nottingham, England). The purity of $E_2$ and EB[1] was checked by thin-layer chromatography. 9-Hydroxyellipticine chlorohydrate was kindly supplied by Le Pecq and Paoletti (Villejuif, France; Le Pecq et al., 1974); 2 mg/ml solutions of the dyes were made in TE buffer and stored at 4 °C in the dark. *Escherichia coli* DNA labeled in vivo by $^{32}$P was purified according to Marmur (1963), then centrifuged in a CsCl gradient, and eventually sonicated.

Calf thymus DNA (type 1) was purchased from Sigma, poly(adenylic acid) (poly(A)) from Calbiochem, and phosphocellulose (P 11) from Whatman. Phosphocellulose was treated before use as described (Burgess, 1969). DNA or poly(A) was adsorbed on Munktell's cellulose powder (Alberts and Herrick, 1971) and stocked dried at −20 °C.

*Initial Steps.* Frozen calf uteri or fresh uteri of 21 day old rats were cut in small pieces in five volumes of 10 mM Tris-HCl, pH 7.4, 1.5 mM EDTA buffer, and homogenized at 2 °C using a Virtis homogenizer or a glass/glass Potter-Elvehjem homogenizer, respectively. Cytosol was separated from particulate fractions by centrifugation for 70 min at 250 000*g*, diluted with TE buffer down to 2 mg/ml of protein, and then incubated at 2 °C with 2 or 3 nM $^3HE_2$ for ≥ 90 min. TE buffer or 10 mg of cellulose powder, containing or not containing DNA, was incubated batchwise with labeled cytosol under linear stirring (60 agitations/min) at 25 or 2 °C.

*Measure of the $E_2R$–Polyanion Complexes.* The binding of $E_2R$ to *soluble* DNA was assayed according to the comigration of [$^3$H]estradiol with DNA by using either molecular sieving on Sepharose 4B (Musliner and Chader, 1972) or ultracentrifugation in a sucrose gradient (André and Rochefort, 1973). As shown previously, $^3HE_2$ did not comigrate with DNA when the receptor was denatured by heating or occupied by nonradioactive estradiol. By using [$^{32}$P]DNA we confirmed that the cosedimentation of $^3HE_2$ with DNA was not due to irreversible aggregates of the receptor, since the $E_2R$ complex was completely liberated as an 8S peak after DNA hydrolysis by DNase (André and Rochefort, 1973a). The receptor in-

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[1] Abbreviations used are: EB, ethidium bromide; $E_2$, estradiol; R, estrogen receptor; $E_2R$, estrogen receptor complex; poly(A), poly(adenylic acid); EDTA, ethylenediaminetetraacetic acid disodium salt; TE buffer, 10 mM Tris, HCl, pH 7.4, 1.5 mM EDTA; EB/DNA, molar ratio of the concentration of total EB to the concentration of DNA phosphate; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; OD, optical density; POPOP, 1,4-bis[2-(5-phenyloxazolyl)]benzene; PPO, 2,5-diphenyloxazole.

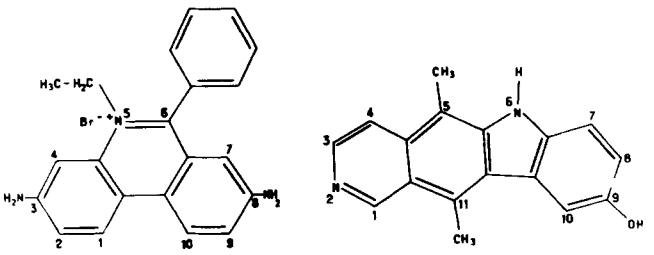

FIGURE 1: Structure of ethidium bromide (left) and 9-hydroxyellipticine (right).

teraction with DNA was also evaluated using a batchwise DNA-cellulose assay (André and Rochefort, 1975). After interaction, the cellulose powder was centrifuged at 2000*g* for 2 min, washed twice with 5 ml of TE buffer, and then counted for radioactivity. The amount of the $E_2R$-DNA complex was deduced from the difference of radioactivity retained by the DNA-cellulose and that retained by the cellulose. The fraction of the input receptor retained by DNA-cellulose and cellulose was 41 ± 18% and 2.8 ± 2%, respectively (mean of 11 experiments ±1 SD). The validity of this technique has been previously demonstrated (André and Rochefort, 1975). More particularly, it was shown that the receptor was responsible for the $^3HE_2$ binding to DNA and that the formation of the complex obeyed a reversible equilibrium. In addition, the increase of radioactivity bound to the powder was correlated to the decrease of estradiol sites in the cytosol. Since some DNA was desorbed during incubation, results were reported per μg/DNA measured after interaction and washings. Bindings to other polyanions-cellulose powders were measured similarly.

*Measure of the Dye Concentrations.* Free EB was assayed by absorption at 480 nm ($\epsilon_M$ = 5450). The total bound EB (intercalated + external) was measured by spectral shift (Waring, 1965). The intercalated EB was evaluated by spectrofluorimetry, using 580 nm for maximal emission and 365 nm for excitation (Le Pecq and Paoletti, 1967). These conditions were suitable with or without cytosol. 9-Hydroxyellipticine was assayed by spectrophotometry at 305 nm ($\epsilon_M$ = 34 924).

*Miscellaneous.* The estradiol-receptor complexes, free or bound to soluble DNA, were assayed by the charcoal-dextran method (Rochefort and Baulieu, 1971). One volume of cytosol and one volume of cold dextran (0.05% w/v) coated charcoal (0.5% w/v) suspension were incubated overnight at 2 °C with stirring. The mixture was then centrifuged 2000*g* for 10 min and an aliquot of the supernatant was counted for radioactivity. The nonspecific binding was evaluated in parallel with a 1000-fold excess of nonradioactive estradiol. The concentration of protein was determined by the method of Hartree (1972) or by absorption at 280 and 260 nm. Soluble DNA was assayed according to Burton and was detected by ultracentrifugation or molecular sieving analysis by the $^{32}P$ radioactivity. Adsorbed DNA was estimated by absorption of the 0.3 N $HClO_4$ hydrolysate (Meijs and Schilperoort, 1971) at 260 nm after an eventual ethanolic extraction of EB. Poly(A) was assayed at 260 nm either directly in TE buffer (1 $OD_{260}$ = 33 μg) or after hydrolysis in $HClO_4$ 0.3 M (1 $OD_{260}$ = 27 μg) for adsorbed poly(A). $^3HE_2$, either soluble or bound to cellulose, was counted in 10 ml of scintillator (POPOP 0.01% w/v-PPO 0.3% w/v in toluene) containing 3 ml of ethanol, giving an efficiency of 20%, as evaluated by external standards. In double labeling experiments, ethanol was replaced by Triton X-100 (5 ml). The contribution of $^{32}P$ in the $^3H$ channel varied from 7 to 17% and was systematically corrected.

## Results

*(1) Inhibition of the $E_2R$ Binding to Adsorbed DNA.* The presence of EB during the incubation of cytosol with DNA-cellulose markedly reduced the binding of $E_2R$ to adsorbed DNA (Figure 2). The binding of $E_2R$ to DNA reached a plateau after ≃30 min of incubation at 25 °C, with or without EB. The effect of increasing concentrations of the dye added simultaneously to DNA and cytosol was then evaluated at equilibrium. The radioactivity retained by DNA-cellulose diminished progressively, whereas that bound to cellulose was constant. The drug did not modify the concentration of the

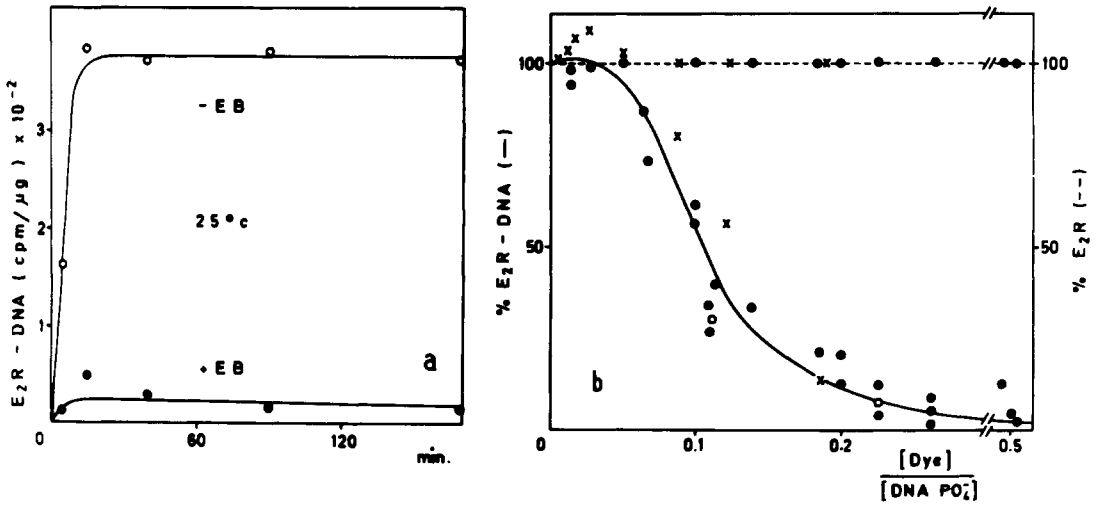


FIGURE 2: Inhibition of the binding to adsorbed DNA. Calf uterine cytosol containing $^3HE_2$ (2 nM) was incubated at 25 °C with either 10 mg of DNA-cellulose containing 6 μg of DNA or 10 mg of cellulose in the presence of increasing concentrations of EB. The $E_2R$-DNA complexes and DNA remaining bound after washing were assayed as described under Experimental Procedure. (a) Time course of the binding of receptor to DNA obtained in the presence of EB (2.6 μg/ml) (●) or in its absence (○). (b) Effect of increasing concentrations of the dye at equilibrium (1 h): the receptor-DNA complexes (—) were assayed for varying concentrations of dye. The stability of the $E_2R$ sites was controlled on a cytosol incubated separately with EB (- - -) using charcoal adsorption. The experiments were performed with EB in the presence (○) or absence (●) of 0.1 M NaCl or with 9-hydroxyellipticine (X). Results of eight experiments are represented; for each of them the 100% value (from 300 to 3000 cpm/μg of DNA) was obtained without dye.

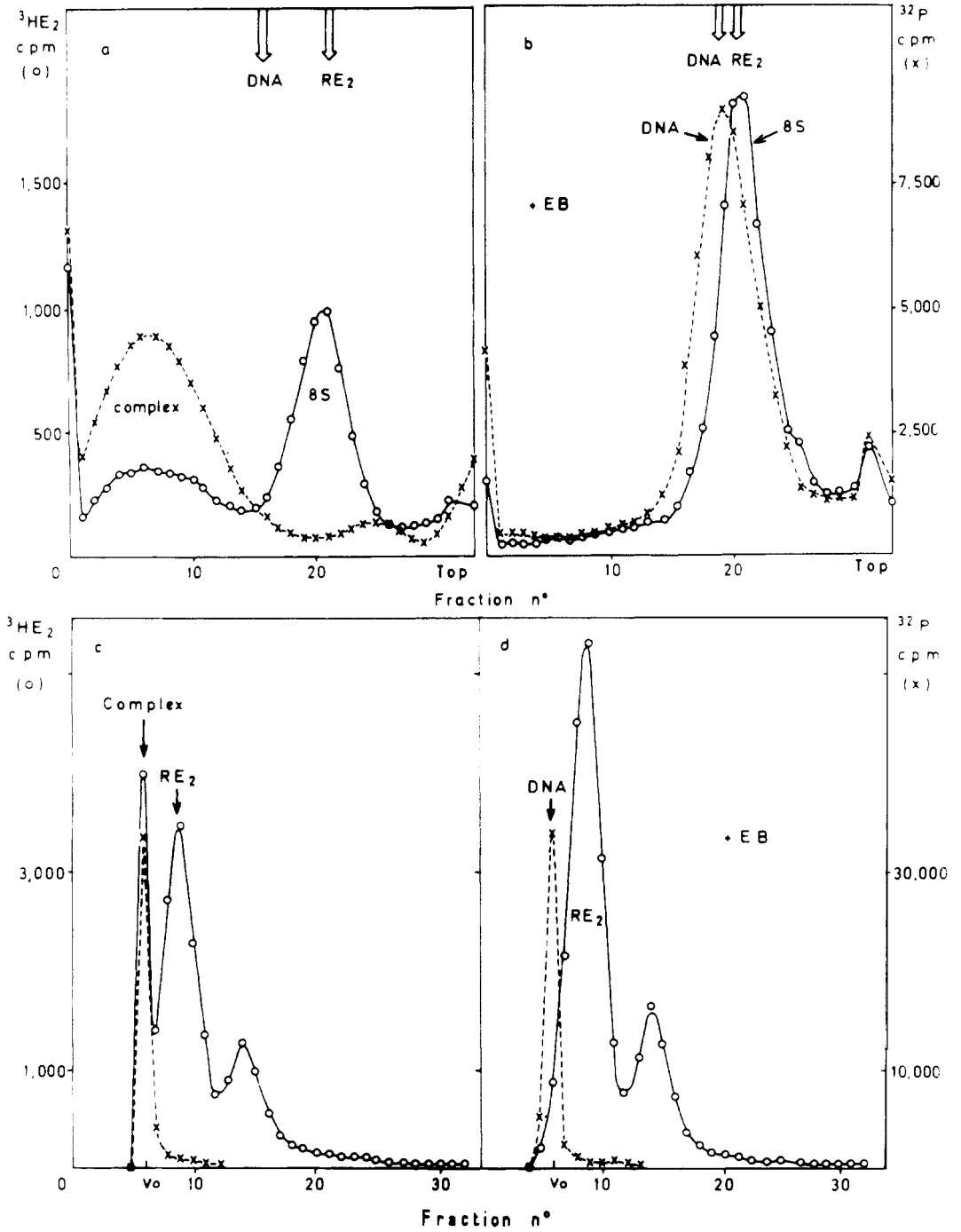


FIGURE 3: Inhibition of receptor binding to soluble DNA. Sucrose gradient ultracentrifugation (a and b): rat uterine cytosol (2 mg/ml of protein) labeled with $^3HE_2$ was treated (b) or not (a) by EB and then incubated for 3 h at 2 °C with *E. coli* [$^{32}$P]DNA (SA: 25 000 cpm/$\mu$g). Final concentrations of EB and DNA were, respectively, 20 and 10 $\mu$g/ml. Aliquots of the incubates were then ultracentrifuged in a 5–20% sucrose gradient for 15 h at 32 000 rpm using an SW 50 rotor. Arrows represent peaks of [$^{32}$P]DNA or $^3HE_2R$ that were run in separate buckets during the same experiment. Sepharose 4B chromatography (c and d): calf uterine cytosol (2 mg/ml of protein) labeled with $^3HE_2$ was incubated for 2 h at 2 °C with *E. coli* [$^{32}$P]DNA (SA: 11 000 cpm/$\mu$g) with (d) or without (c) EB. Final concentrations of EB and DNA were, respectively, 1 and 6 $\mu$g/ml. The incubate (0.4 ml) was then analyzed in a 20-ml column of Sepharose 4B and equilibrated with TE buffer at a flow rate of 12 ml/h according to Musliner and Chader, 1972. Fractions (1.2 ml) were collected and counted for $^{32}$P and $^3$H radioactivity.

available estrogen receptor sites in the presence or absence of DNA, as measured in parallel by charcoal assay. By employing [$^{32}$P]DNA we assessed that EB did not decrease the amount of DNA retained on the cellulose. This indicated that EB inhibited the $E_2R$–DNA interaction. Furthermore, 50% inhibition was observed with a molar ratio (EB/DNA) of 0.1 and total inhibition with a ratio of 0.25. 9-Hydroxyellipticine displayed similar activity. The interaction of the receptor with DNA in the absence of estradiol, although lower than in its presence, was also inhibited by EB (not shown). The same inhibition was obtained whether the drug had been first incubated with cytosol or with DNA, or at the moment of the mixing. Conversely, once the $E_2R$ was bound to DNA, either at 2 or 25 °C, less than 20% of $E_2R$ could be displaced during a 1-h incubation at 25 °C with high EB/DNA ratio ($\simeq$8). The fact that the fluorescence of the intercalated drug was similar, whether DNA had been preincubated or not with the cytosol, suggests that the majority of soluble DNA was accessible to

TABLE I: Reversibility of the Inhibition.[a]

| Pretreatment | $E_2R$ DNA Complexes (cpm) |
|---|---|
| Cytosol | |
| (1) Control | 13 900 |
| (2) EB | 0 |
| (3) Sephadex G-25 | 7 500 |
| (4) EB, Sephadex G-25 | 6 700 |
| Cellulose ± DNA | |
| (5) EB, TE buffer, wash | 1 200 |
| (6) EB, soluble DNA, wash | 14 100 |

[a] The following pretreatments were applied to either calf uterine cytosol labeled with 3 nM $^3HE_2$ or cellulose containing or not containing DNA. (A) EB 25 μg/ml was added (2) or not (1) to the cytosol; (B) Cytosols with (4) or without (3) EB were passed through a Sephadex G-25 column and the void volumes were collected. The cytosols (1, 2) and void volumes (3, 4) were made 1 mg/ml of protein and incubated with cellulose and DNA cellulose for 1 h at 25 °C. (C) Cellulose and DNA-cellulose were pretreated by EB (EB/DNA ratio of 2) for 10 min at 25 °C, then incubated overnight at 2 °C with TE buffer containing (6) or not (5) an excess of soluble DNA (270 μg/ml), subsequently washed twice, and finally incubated with cytosol (1 mg/ml of protein) containing $^3HE_2$ for 1 h at 25 °C. In all cases, the $E_2R$-DNA complexes were assayed as described under Experimental Procedure.

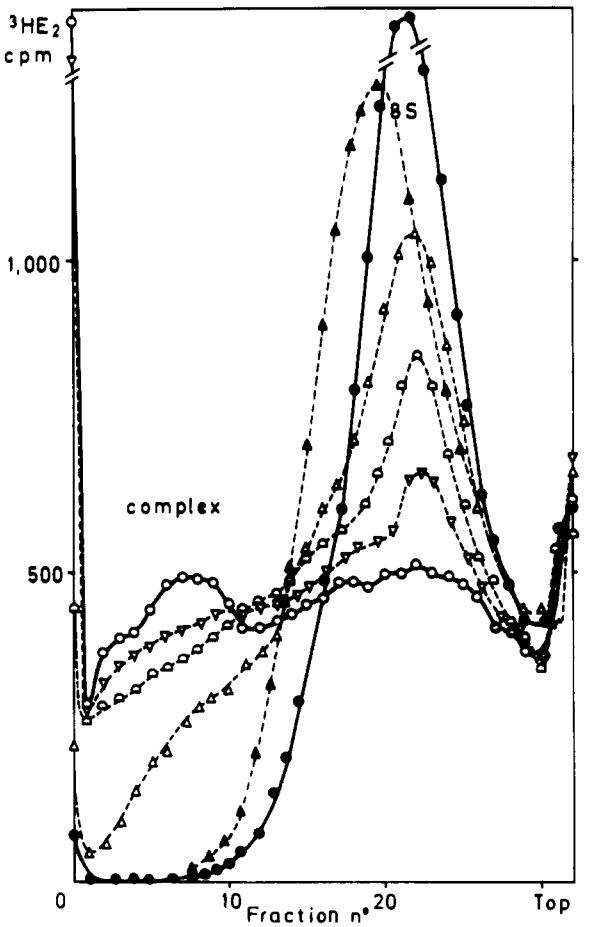


FIGURE 4: Inhibition of the $E_2R$-DNA interaction by increasing concentrations of EB. Calf uterine cytosol was initially incubated for 2 h at 2 °C with 3nM $^3HE_2$ and subsequently for 2 h at 2 °C with DNA or TE buffer with or without EB. The EB treated (- - -) and control (—) samples were centrifuged in 5-20% sucrose gradient as described in Figure 3. Cytosol containing DNA (180 μg/ml) and no EB (○) or 6.8 μg/ml (▽) or 13.6 μg/ml (⌓) or 20.3 μg/ml (△) of EB; cytosols without (●) or with (▲) 20.3 μg/ml of EB.

EB, but does not exclude a lack of accessibility of the drug for the receptor binding sites of DNA. However, these results agree with the assumption that the association rate of EB to DNA is faster than that of $E_2R$ to DNA and that the dissociation rate of the $E_2R$-DNA complex is low, as already indicated by the resistance of this complex to diluted buffer (André and Rochefort, 1975).

(*2*) *Inhibition of the $E_2R$ Binding to Soluble DNA.* Following incubation of the DNA cytosol mixture at 2 °C, inhibition was evaluated using Sepharose chromatography and sucrose gradient ultracentrifugation (Figure 3). In both cases EB decreased the amount of the DNA-$E_2R$ complex and increased that of the free receptor. Moreover, in the absence of EB the *s* value of DNA was increased after incubation with uterine cytosol (Figure 3a). Nonreceptor proteins were probably responsible for this displacement, which also occurred with cytosol containing unfilled receptor, spleen cytosol, or uterine cytosol heated to 70 °C. The drug inhibited this displacement, suggesting that it also prevented the binding of other cytosol proteins to DNA. When EB was added separately to a DNA solution or to uterine cytosol, it decreased the *s* value of DNA (Figure 3a,b) and increased that of $E_2R$ (Figure 4). Conversely, in the presence of DNA the migration of the free $E_2R$ was not modified by the drug. These results suggest that under our experimental conditions EB was preferentially bound to DNA in a cytosol-DNA mixture. They also indicate that EB did not transform the 8S receptor into a 4S subunit unable to interact with DNA (André and Rochefort, 1973b). The classical activating treatment of the cytosol receptor at 25 °C was not employed in these experiments, since it resulted in the aggregation of the receptor making it difficult to distinguish from its interaction with DNA. However, it was assessed that binding equilibrium between DNA and receptor had been reached at 2 °C in the sucrose gradient experiments.

(*3*) *Reversibility of the Inhibition.* Further experiments indicated that neither the estrogen receptor nor DNA were irreversibly inactivated by the drug. When the adsorbed DNA concentration was increased with constant EB (2 μg/ml) and cytosol protein (2 mg/ml) concentrations, the total inhibition observed with 5 μg DNA was decreased to 70 and 0 per cent for DNA concentration values of 10 and 15 μg/ml respectively. In addition, in a cytosol which had been pretreated by EB in order to obtain a total inhibition, the receptor was again able to bind DNA after removal of the dye by Sephadex G 25 chromatography (Table I). Moreover, it is noticeable that the DNA binding ability of the receptor was decreased by gel filtration suggesting that low molecular weight compound of the cytosol favored the receptor-DNA interaction. These experiments indicated the absence of irreversible alteration of $E_2R$ by the drug. Moreover, DNA cellulose which had been treated by effective concentrations of EB fully recovered its ability to bind the $E_2R$ when EB was removed with an excess of soluble DNA. We concluded that the inhibition of $E_2R$-DNA interaction by EB was reversible and probably due to intercalation between DNA bases although the possibility of a reversible competition of EB on the DNA binding site of the receptor could not be totally excluded.

(*4*) *Correlation between Intercalation and Inhibition.* Using soluble DNA, quantitative studies were performed to determine whether the intercalation or ionic binding of the dye was responsible for the inhibition of steroid-receptor binding to DNA. After incubation of increasing concentrations of EB

TABLE II: Effect of EB on the $E_2R$ Binding to Polyanions.[a]

| Expt. No. | Powder | Phosphate (μequiv) | $E_2R$ Bound (cpm/pellet) −EB | $E_2R$ Bound (cpm/pellet) +EB | EB (μmol) |
|---|---|---|---|---|---|
| 1 | Phosphocellulose | 185 | 740 | 890 | 23 |
| | Phosphocellulose | 1 850 | 1 340 | 1 720 | 23 |
| | Phosphocellulose | 18 500 | 8 700 | 11 180 | 23 |
| | Cellulose | 0 | 600 | 830 | 23 |
| | DNA-cellulose | 60 | 25 200 | 1 470 | 23 |
| 2 | Poly(A)-cellulose | 50 | 740 | 1 720 | 12 |
| | Cellulose | 0 | 210 | 190 | 12 |
| | DNA-cellulose | 40 | 3 140 | 380 | 12 |
| 3 | Poly(A)-cellulose | 50 | 1 400 | 2 300 | 5.3 |
| | Poly(A)-cellulose | 50 | 1 400 | 2 700 | 10.6 |
| | Poly(A)-cellulose | 50 | 1 400 | 2 800 | 16.2 |
| | Poly(A)-cellulose | 50 | 1 400 | 3 700 | 27.1 |

[a] Calf uterine cytosol, with or without EB, was incubated for 1 h at 25 °C with cellulose containing or not containing polyanions. The $E_2R$ bound to polyanions was assayed as described under Experimental Procedure. The phosphate concentrations were estimated according to the dilution of the phosphocellulose Whatman P11 by Munktell's cellulose or deduced from the 260-nm absorption for poly(A) and DNA-cellulose.

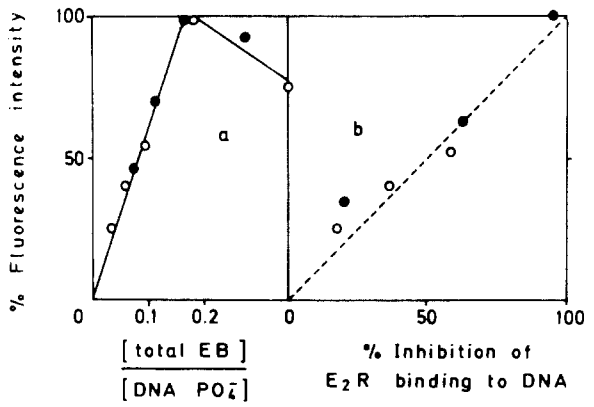


FIGURE 5: Fluorescence of the intercalated EB and its correlation with the inhibition of the $E_2R$-DNA interaction. During the experiment shown in Figure 4 (○) or a similar experiment performed with a lower DNA concentration (136 μg/ml) (●) the fluorescence of the intercalated EB was measured (a) and correlated with the inhibition of the $E_2R$-DNA interaction (b). The fluorescence intensity of the cytosol containing DNA and increasing concentrations of EB was measured as under experimental procedure. The low fluorescence observed in the control cytosols containing EB but no DNA was systematically subtracted. The highest corrected fluorescence was taken as 100%. The binding of $E_2R$ to DNA was assayed after ultracentrifugation in a sucrose gradient as in Figures 3 and 4. Its percentage of inhibition was defined according to the relative increase of the radioactivity in the 8S fraction assuming a 0% inhibition in the absence of EB and a 100% inhibition in the absence of DNA. Inhibition (%) = [(8S cpm(cytosol, DNA, EB) − 8S cpm(cytosol, DNA))/(8S cpm(cytosol) − 8S cpm(cytosol, DNA))] × 100.

with a constant cytosol receptor and DNA content, the degree of $E_2R$-DNA binding was evaluated by ultracentrifugation in a sucrose gradient. The concentration-dependent inhibition of the $E_2R$-DNA interaction by EB was demonstrated by a progressive decrease of the radioactivity bound to DNA in the 12-20S region and a resulting liberation of the free $E_2R$ (Figure 4). Total inhibition was shown with high EB/DNA ratios (Figure 3b). We found it more convenient to evaluate the $E_2R$-DNA interaction indirectly according to the height of the 8S complex, since the migration of the $E_2R$-DNA complex varied according to the EB concentrations, whereas that of the free $E_2R$ complex was not altered by the drug. Since the drug bound to the ionic site is not fluorescent, the intercalated EB was evaluated according to the increase of fluorescence intensity (Le Pecq and Paoletti, 1967) (Figure 5a). For EB/DNA ratios ≤ 0.17, the fluorescence was approximately proportional to the total EB concentration. Under these conditions, the large majority of the bound EB was intercalated. The degree of inhibition of the $E_2R$-DNA binding being approximately proportional to the fluorescence intensity of EB suggested that the inhibition was correlated to the amount of intercalated drug (Figure 5b). These results are consistent with those obtained using DNA-cellulose (Figure 2b). In this case, a 50% inhibition was also achieved with EB/DNA ratios of ≃0.1. The presence of 0.1 M NaCl, which is known to decrease the external ionic binding of the drug to DNA (Le Pecq and Paoletti, 1967), did not modify the degree of inhibition (Figure 2b). These data strongly suggest that the intercalation of the drug between DNA bases prevents the interaction of the estrogen receptor with DNA.

(*5*) *Polyanion Specificity*. When using cellulose bearing different phosphate concentrations (Table II), the amount of $E_2R$ bound/μequiv of phosphate was much lower with phosphocellulose or poly(A)-cellulose than with double-stranded DNA. EB which interacts with these three polyanions inhibited the receptor binding to DNA-cellulose and increased that to poly(A)- or phosphocellulose. The mechanism of the EB induced increase of receptor binding to poly(A)-cellulose has not been studied, although poly(A) was shown to partly retain EB after incubation with cytosol. We concluded that DNA did not interact with the receptor exclusively through their phosphate groups. In addition competitive experiments (Table III) performed with soluble polynucleotides indicated that double-stranded DNA was more efficient at competing with adsorbed DNA for $E_2R$ binding than denatured DNA or poly(A). The affinity of polynucleotides for $E_2R$ appeared related to the content of double helices, since poly(A) was totally inefficient, whereas denatured DNA that contains short double-stranded regions was slightly competitive. The fact that $E_2R$ binding to denatured DNA was prevented by EB supports this assumption. These results indicate that the binding of $E_2R$ is

TABLE III: Competition between Soluble Polynucleotides and Adsorbed DNA:[a]

| | | $E_2R$ Bound (cpm/μg of adsorbed DNA) | |
|---|---|---|---|
| | μg/0.4 ml | Expt. 1 | Expt. 2 |
| Control | 0 | 1680 | 1300 |
| Native calf thymus DNA | 20 | 630 | - |
| | 25 | - | 450 |
| | 50 | - | 320 |
| Denatured[b] calf thymus DNA | 20 | 1350 | - |
| | 25 | - | 820 |
| | 50 | - | 720 |
| Poly(A) | 20 | 1760 | 1500 |

[a] TE buffer (0.1 ml) with or without polynucleotides was mixed in duplicate with 10 mg of DNA-cellulose and 10 mg of cellulose. Calf uterine cytosol (0.3 ml) previously labeled with $^3HE_2$ (5 nM) was immediately added and the mixture incubated for 1 h at 25 °C. The $E_2R$ bound to DNA and adsorbed DNA (≃6 μg) were then measured in duplicate as described. [b] TE buffer containing calf thymus DNA (200–500 μg/ml) was heated for 10 min at 100 °C and rapidly cooled in ice (optical increment: 33%).

more important with double-helical DNA than with polymers bearing the same concentration of phosphate charges.

## Discussion

The reported data indicate that EB inhibits the binding of $E_2R$ to double-stranded DNA by intercalating between DNA bases pairs. The effects of intercalating drugs and the marked differences observed between the efficiency of several polyanions to bind $E_2R$ (Tables II and III) lead us to conclude that the binding of $E_2R$ to DNA cannot only be interpreted in terms of simple electrostatic interactions with phosphate charges. These results also suggest that $E_2R$ preferentially interacts with double-helical nucleic acids. The mechanism of the inhibition due to EB is unknown; the intercalated part of EB, and not its phenyl ring located in a DNA groove, is probably responsible for this inhibition, since 9-hydroxyellipticine displayed similar efficiency with only one methyl group in each groove (Le Pecq, personal communication). Whether the intercalated dye competes with the receptor for the same site or prevents indirectly its binding in unwinding the double-helical DNA remains to be precised. Low concentrations of EB have been shown to inhibit the activities of enzymes that bind to DNA (Richardson and Parker, 1973; Hirsham, 1971), indicating that the consequence of DNA intercalation is not specific for the receptor. Further experiments with purified receptor are therefore needed to specify whether the interaction of the receptor with DNA is direct or mediated by other components of the cytosol. The reported inhibition applies to a DNA receptor interaction that is reversible, of low affinity, and large binding capacity, and that displays no DNA sequence specificity. The possibility of another interaction of the receptor with specific nucleotide sequence is, however, not excluded by the present studies. The fact that these intercalating drugs also inhibit the nuclear translocation of estrogen (in preparation) and aldosterone (Edelman, 1975) receptors favors the hypothesis that accessible and double-helical DNA can be at least a part of the physiological nuclear acceptor for the hormone–receptor complex.

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## References

Alberts, B., and Herrick, G. (1971), *Methods Enzymol. 21,* 198.
Andre, J., and Rochefort, H. (1973a), *FEBS Lett. 29,* 135.
Andre, J., and Rochefort, H. (1973b), *FEBS Lett. 32,* 330.
Andre, J., and Rochefort, H. (1975), *FEBS Lett. 50,* 319.
Burgess, R. R. (1969), *J. Biol. Chem. 244,* 6160.
Burton, K. (1968), *Methods Enzymol. 12B,* 163.
Edelman, I. S. (1975), *J. Steroid Biochem. 6,* 147.
Hartree, E. F. (1972), *Anal. Biochem. 48,* 422.
Hirscham, S. Z. (1971), *Science, 173,* 441.
Jensen, E. V., and De Sombre, R. (1973), *Science 182,* 126.
Le Pecq, J. B., and Paoletti, C. (1967), *J. Mol. Biol. 27,* 87.
Le Pecq, J. B. (1971), *Methods Biochem. Anal. 20,* 41.
Le Pecq, J. B., Nguyen Dat-Xuong, Gosse, C., and Paoletti, C. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 5078.
Marmur, J. (1963), *Methods Enzymol. 6,* 726.
Meijs, W. H., and Schilperoort, R. A. (1971), *FEBS Lett. 12,* 166.
Musliner, T. A., and Chader, G. J. (1972), *Biochim. Biophys. Acta 262,* 256.
Richardson, J. P., and Parker, S. R. (1973), *J. Mol. Biol. 78,* 715.
Rochefort, H., and Baulieu, E. E. (1971), *Biochimie 8,* 893.
Toft, D. (1973), *Adv. Exp. Med. Biol. 36,* 36.
Waring, M. J. (1965), *J. Mol. Biol. 13,* 269.