Also, the wide difference in the slope of the curves of mouse satellite DNA and guinea pig satellite DNA I indicates that the latter might contain sequences of DNA with rather different rates of reassociation. The presence of sequences with different rates of reassociation in satellite DNAs could be in agreement with the fact that in general a complete sequence matching of the complementary strands of satellite DNAs is not obtained upon reassociation after denaturation.

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References

Britten, R. J. (1969), *Yearbook Carnegie Instn. 68*, 332.
Britten, R. J., and Kohne, D. E. (1966), *Yearbook Carnegie Instn. 65*, 78.
Britten, R. J., and Kohne, D. E. (1968), *Science 161*, 529.
Corneo, G., Ginelli, E., and Polli, E. (1968a), *J. Mol. Biol. 33*, 331.
Corneo, G., Ginelli, E., Soave, C., and Bernardi, G. (1968b), *Biochemistry 7*, 4373.
Eigner, J., and Doty, P. (1965), *J. Mol. Biol. 12*, 549.
Flamm, W. G., McCallum, M., and Walker, P. M. B. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 1729.
Flamm, W. G., Walker, P. M. B., and McCallum, M. (1969a), *J. Mol. Biol. 40*, 423.
Flamm, W. G., Walker, P. M. B., and McCallum, M. (1969b), *J. Mol. Biol. 42*, 441.
Jensen, R. H., and Davidson, N. (1966), *Biopolymers 4*, 17.
Mandel, J. D., and Hershey, A. D. (1960), *Anal. Biochem. 1*, 66.
Marmur, J. (1961), *J. Mol. Biol. 3*, 208.
Marmur, J., and Doty, P. (1962), *J. Mol. Biol. 5*, 109.
Nandi, U. S., Wang, J. C., and Davidson, N. (1965), *Biochemistry 4*, 1687.
Polli, E., Ginelli, E., Bianchi, P., and Corneo, G. (1966), *J. Mol. Biol. 17*, 309.
Schildkraut, C. L., Marmur, J., and Doty, P. (1962), *J. Mol. Biol. 4*, 430.
Vinograd, J., and Hearst, J. E. (1962), *Progr. Chem. Org. Natl. Prod. 20*, 395.
Walker, P. M. B., and McLaren, A. (1965), *J. Mol. Biol. 12*, 394.
Waring, M., and Britten, R. J. (1966), *Science 154*, 794.
Wetmur, J. G., and Davidson, N. (1968), *J. Mol. Biol. 31*, 349.

Interactions of Hormonal Steroids with Nucleic Acids. III. Role of Polymer Structure*

Chev Kidson, Ann Thomas,† and Paul Cohen‡

ABSTRACT: The role of polymer structure in the guanine-specific binding of progesterone, testosterone, and estradiol under equilibrium conditions has been examined by the use of particular nucleic acids. Possible steroid associations with chain-terminal guanine residues have been excluded by the following observations: binding occurs with circular, single-stranded *M13* and *φX174* DNAs; binding occurs with denatured T_7 DNA lacking terminal guanine residues; the numbers of binding sites on sheared and unsheared *Pseudomonas aeruginosa* DNA having a 50-fold difference in average chain length are not greatly different; the fluorescence quantum yield and fluorescence emission spectrum of poly-7-methylguanylic acid are unchanged in the presence of estradiol.

These data together support the model of steroid association internally along polynucleotide chains.

A number of hormonal steroids bind to ribo- and deoxyribopolynucleotides under equilibrium conditions in aqueous buffer (Cohen and Kidson, 1969a). There is a specific requirement for guanine residues, except in the case of estradiol, which will also bind to inosine (Cohen and Kidson, 1969b). The observation that two functional groups of each steroid are necessary for binding to guanine residues in polyguanylic acid, the fact that these functional groups can act as proton donors or acceptors and the finding that slightly protic solvents have minimal effects on the binding of the steroids to polynucleotides suggest that two hydrogen bonds are formed in each case between functional groups of steroid and purine (Cohen *et al.*, 1969), although hydrophobic forces are undoubtedly involved in these associations.

Several attributes of polynucleotide structure essential to the binding of steroids have already been ascertained.

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Binding sites must be single stranded or at least partly disordered, as in denatured DNA (Cohen and Kidson, 1969b). At the same time, conditions associated with increased base stacking (high salt) were found to enhance the binding of progesterone and testosterone but not of estradiol (Cohen and Kidson, 1969b), experimental observations which were found by model building to be consistent in each case with association of steroid with a short nucleotide sequence. Binding was eliminated by protonation of guanine residues in polyguanylic acid, when partial disruption of helical structure occurred (Cohen *et al.*, 1969). The finding that the number of steroid binding sites corresponding to maximum binding constants was less than 1/10,000 nucleotide residues in polyguanylic acid or in denatured DNA has been interpreted as reflecting the strict polymer conformational requirements (Cohen *et al.*, 1969). However, an important alternative possibility is that these binding sites, corresponding to free energy of binding of 7–10 kcal/mole of steroid bound, may reflect steroid–guanine associations at the ends of polynucleotide chains. In the present studies, the role of polymer structure is investigated further, with the conclusions that the ends of polynucleotide chains are not necessary for steroid binding and that binding to the ends of chains is indeed unlikely.

Materials and Methods

Ribopolymers. Poly G was obtained from Miles Laboratories, Inc., and the concentration determined spectrophotometrically on the basis of ϵ_{max} 9.10^3 at 252 mμ. Poly-7-methylguanylic acid was prepared by methylation of poly G with dimethyl sulfate, by the method of Michelson and Pochon (1966). The resulting product showed a 6-mμ red shift in λ_{max}, from 252 to 258 mμ.

DNA was isolated from *Escherichia coli* and *Pseudomonas aeruginosa* by phenyl methods (Kidson, 1966), using sodium triisopropylnaphthalenesulfonate as detergent (Parish and Kirby, 1966). Before isolation, *E. coli* DNA was generally labeled with [^{3}H]thymidine (20 Ci/mmole, New England Nuclear Corp.), for use as a standard in the exonuclease I assay. Bacteriophage T_7 was grown according to Thomas and Abelson (1966) and T_7 DNA was isolated from the intact phage after banding in CsCl, essentially by the method of Davison and Freifelder (1962). Sterile buffers and glassware were used at all times and care was taken to avoid shearing by the use of wide-bore pipets and microsyringes. Bacteriophage $\phi X174$ was grown according to Sinsheimer (1959a) and DNA isolated by the method of Sinsheimer (1959b). DNA from bacteriophage *M13*, and previously characterized ^{3}H-labeled circular and ^{3}H-labeled mixed linear and circular DNA preparations from bacteriophage $\phi X174$, were the generous gifts of Dr. Regis Kelly. DNA was stored at −20° or at 4° in HMP[1] buffer containing 0.75 M NaCl. Concentration was determined spectrophotometrically on the native form on the basis $\epsilon^{1\%}_{1cm}$ 200 at 260 mμ.

Denaturation and Shearing of DNA. As indicated, DNA was denatured either by heating the HMP buffer at 100° for 10 min, followed by rapid cooling on ice, or by alkali at pH 12.8 for 5 min, followed by neutralization with HCl. Native *Ps. aeruginosa* DNA was sheared where indicated, before denaturation, by sonication for 2 min with a Branson Sonifier at setting 3.

Sedimentation of DNA. $s^0_{20,w}$ values were obtained with 5–20 μg of DNA in HMP–0.75 M NaCl, using the Spinco Model E analytical ultracentrifuge fitted with ultraviolet optics. The homogeneity of size of denatured T_7 DNA was assessed by analytical ultracentrifugation at pH 12.8 and by centrifugation in a linear, 5–25% sucrose density gradient, pH 12.8, in a Spinco Model L2 preparative ultracentrifuge. The circularity of *M13* and $\phi X174$ DNAs was assessed before and after incubation with exonuclease I, using the above-mentioned ^{3}H-labeled $\phi X174$ DNAs as markers. Exonuclease I was the gift of Dr. I. R. Lehman; incubation was carried out according to Lehman (1963), and the mixture extracted was phenol or $CHCl_3$ before centrifugation to remove enzyme protein. Certain controls were treated with pancreatic deoxyribonuclease (electrophoretically purified, Worthington) as indicated.

Radiochemicals. The following radioactive steroids of high specific activity were obtained from the New England Nuclear Corp.: [1,2-^{3}H]testosterone (50 Ci/mmole), [6,7-^{3}H]estradiol-17β (50 Ci/mmole), [1,2-^{3}H]progesterone (50 Ci/mmole). Radiochemical purity was checked by thin-layer chromatography in hexane–ethyl acetate–methanol (9:9:2, v/v). Radioactivity was assessed using a Packard radiochromatogram scanner.

Spectroscopy. Ultraviolet spectra were measured with a Zeiss PMQ II or a Carey Model 14 spectrophotometer. Optical rotatory dispersion spectra were recorded with a JASCO spectropolarimeter (ORD-UV5 Model) using a cell of 1-cm path length. Fluorescence spectra and relative fluorescence measurements were recorded with an Aminco-Bowman spectrofluorometer.

Measurement of Binding Parameters. Except of fluorescence measurements, binding data were obtained by equilibrium dialysis, as described previously (Cohen and Kidson, 1969b), using polymethylmethacrylate cells of 100 or 500 μl each side, separated by pretreated Visking dialysis tubing. Binding data have been expressed in terms of nK (M^{-1}) (Cohen and Kidson, 1969b) or have been treated by the method of Scatchard (1949) (Cohen *et al.*, 1969).

Results and Discussion

Binding of Steroids to Circular Single-Stranded DNA. The most straightforward test of the possibility that the ends of polynucleotide chains are necessary for binding steroids is the use of circular DNA. Since steroids require single-stranded regions of DNA for binding, DNA in the form of single-stranded circles was chosen, namely, from coliphages *M13* and $\phi X174$.

BINDING TO *M13* DNA. The circularity of *M13* DNA was assessed as follows. Centrifugation in a linear alkaline CsCl gradient gave a single peak which sedimented just ahead of a ^{3}H-labeled circular $\phi X174$ DNA marker in a parallel gradient (Figure 1). After incubation with exonuclease I, *M13* DNA sedimented in the same position, whereas after incubation with pancreatic DNAse there was a marked change in sedimentation properties (Figure 1). Exonuclease I degrades single-stranded DNA from a free 3′-OH end (Lehman, 1960);

[1] HMP buffer = 0.0025 M Na_2HPO_4–0.005 M NaH_2PO_4–0.001 M Na_2EDTA (pH 6.8).

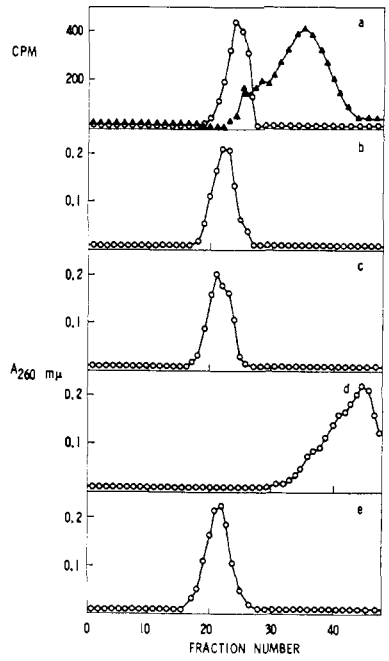

FIGURE 1: Determination of circularity of *M13* DNA. Sedimentation on preformed CsCl density gradients, ρ 1.20–1.35, pH 12.8, at 15° at 46,000 rpm in the Spinco SW50 rotor for 80 min. Absorbancy was read at 260 mμ; radioactive fractions were precipitated with 5% trichloroacetic acid, collected on Millipore filters, and counted by liquid scintillation spectrometry. (a) (—○—) Circular and (—▲—) mixed linear and circular ^{3}H-labeled *φX174* DNA markers, (b) *M13* DNA; (c) *M13* DNA incubated with 4 units of exonuclease I at 37° for 30 min, followed by extraction with phenol, then ether to remove enzyme; (d) *M13* DNA incubated with pancreatic DNase (0.1 μg/ml) for 3 min at 20°, followed by extraction with phenol, then ether; and (e) *M13* DNA recovered from equilibrium dialysis experiment.

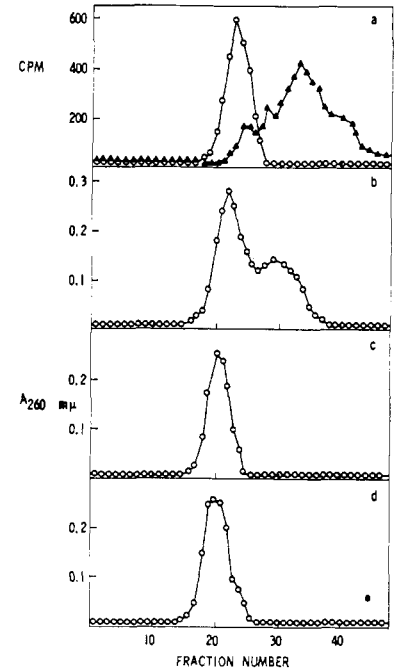

FIGURE 2: Determination of circularity of *φX174* DNA. Sedimentation as in legend to Figure 1. (a) (—○—) Circular and (—▲—) mixed circular and linear, ^{3}H-labeled *φX174* DNA markers; (b) *φX174* DNA; (c) *φX174* incubated with 4 units of exonuclease I at 37° for 30 min, followed by extraction with phenol, then ether; and (d) *φX174* DNA, pretreated with exonuclease I, recovered from equilibrium dialysis experiment.

assay of the preparation used in these experiments with radiolabeled, native and denatured *E. coli* DNA showed the enzyme to be essentially free of endonuclease activity. Resistance of *M13* DNA to exonuclease I may, therefore, be taken as an indication of circularity. Equilibrium dialysis with progesterone, testosterone, and estradiol showed that all three steroids bind to the circular, single-stranded DNA (Table I). Gradient sedimentation of *M13* DNA recovered from the cells after dialysis showed that circularity was not lost during the experiment (Figure 1).

BINDING TO *φX174* DNA. Linear, alkaline CsCl gradient sedimentation of *φX174* DNA showed the presence mainly of circular, but also of some linear forms (Figure 2a), the latter being lost following incubation with exonuclease I (Figure 2b). Equilibrium dialysis showed binding of all three steroids to occur to DNA both before and after treatment with exonuclease I (Table I), with little difference in the respective comparative *nK* values for each steroid. High salt increased the *nK* values (Table I), as reported previously with linear DNA (Cohen and Kidson, 1969b).

Together these data indicate that binding of steroids can occur in the absence of DNA chain ends, implying that the latter are not essential for these associations.

Binding of Steroids to Linear DNA Lacking Terminal Guanine Residues. The binding of steroids to deoxypolynucleotides requires guanine residues (Cohen and Kidson, 1969b). The 5′-terminal residues of T_7 DNA are dA and T, respectively, the corresponding complementary 3′-terminal residues must, therefore, be T and dA (Richardson, 1966). Denatured T_7 DNA is thus a suitable model with which to examine binding to linear DNA molecules lacking terminal guanine residues, provided the strands are essentially intact. The native T_7 DNA isolated in the present experiments was found by analytical centrifugation to be homogeneous (Figure 3a) and to have an $s^0_{20,w}$ = 34.3 S. Analytical centrifugation in alkali gave evidence of some heterogeneity of size of single strands (Figure 3b) but >80% sedimented homo-

TABLE I: Relative Affinities of Steroids for Circular Single-Stranded *M13* DNA and *φX174* DNA.

	nK (M^{-1})			
		φX174 DNA		
		Before Exonuclease I		After Exonuclease I
Steroid	*M13* DNA (HMP)	(HMP)	(HMP–0.5 M NaCl)	(HMP–0.5 M NaCl)
Progesterone	168	240	502	455
Testosterone	87	89	174	180
Estradiol	102	104	125	110

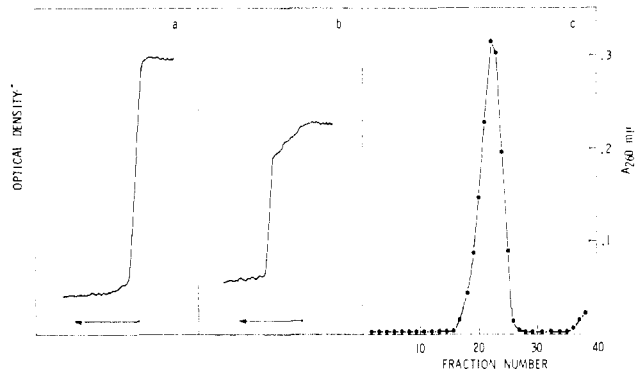

FIGURE 3: Analytical centrifugation of T_7 DNA. (a) Photometric trace of ultraviolet absorption photograph of sedimenting T_7 DNA (14 μg/ml) in HMP–0.75 M NaCl (pH 6.8), 24 min after reaching 34,250 rpm; (b) photometric trace of ultraviolet absorption photograph of sedimenting T_7 DNA (15 μg/ml) in HMP–0.75 M NaCl (pH 12.8) 32 min after reaching 34,250 rpm; and (c) sedimentation of T_7 DNA in a 5–20% sucrose gradient (pH 12.8) containing 1 M NaCl, at 4° at 45,000 rpm for 3 hr in a Spinco SW50 rotor.

geneously with an $s^0_{20,w}$ = 40.0 S. Sedimentation in a linear, alkaline sucrose gradient showed a pattern consistent with homogeneity of size of the strands (Figure 3c). These properties are in agreement with the observations of Davison *et al.* (1964) and of Studier (1965) that a high proportion of the individual strands of the duplex molecules are free of interruptions.

Equilibrium dialysis of denatured T_7 DNA showed binding with progesterone, testosterone, and estradiol (Table II), suggesting that terminal guanine residues are not essential for binding of steroids to linear denatured DNA. Analysis of the binding isotherm for testosterone with denatured T_7 DNA (Figure 4) showed a biphasic curve similar to those reported previously (Cohen *et al.*, 1969), suggesting that binding of the steroid in this case is not dissimilar in principle to that where DNA strand breaks may be presumed to be essentially random (with a proportion of terminal guanine residues). A simplified resolution of the binding isotherm into two components gave numbers of binding sites (Table III) which would correspond, respectively, to one or two sites

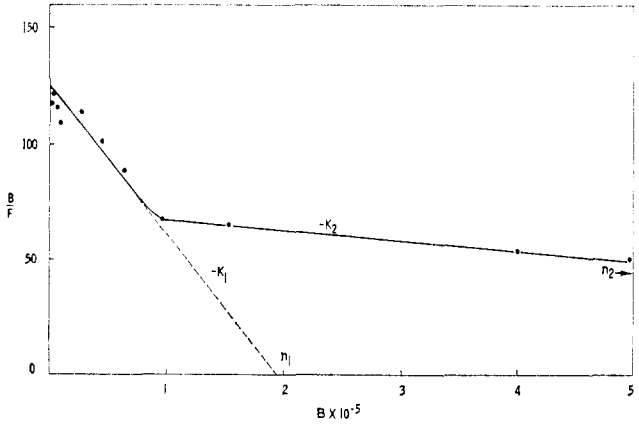

FIGURE 4: Scatchard plot of testosterone binding to denatured T_7 DNA in HMP buffer. Equilibrium dialysis at 4° for 48 hr.

TABLE II: Relative Affinity of Steroids for Denatured T_7 DNA in HMP Buffer.

Steroid	nK (M^{-1})
Progesterone	112
Testosterone	65
Estradiol	44

(*n1*) and 10–20 sites (*n2*) per T_7 DNA molecule. Even the number (*n1*) corresponding to the higher binding constant, *K1*, is greater than could be expected if a high proportion of the few nicks (Figure 3b) present had guanine termini which bound steroid. The magnitude of the higher number, *n2*, corresponding to the lesser binding constant, *K2*, certainly indicated the presence of steroid binding sites situated internally in T_7 DNA chains. Binding of steroid to guanine residues near chain termini cannot be excluded, however, since subterminal guanine residues exist in T_7 DNA (Weiss and Richardson, 1967).

Binding of Steroids to Poly-7-methylguanylic Acid. The binding of steroids to circular single-stranded DNA means that ends of polynucleotide chains are not essential for these associations. The binding of steroids to linear single-stranded DNA lacking guanine termini implies that the latter are not essential for associations with noncircular DNA. However, it is still possible that terminal guanine residues, when they occur, may form preferred binding sites, *e.g.*, in poly G. When the guanine residues in poly G are methylated at position 7, the polymer can fluoresce; the quantum yield is less than that of the monomer (Pochon *et al.*, 1968; Leng *et al.*, 1968), suggesting that emission occurs at the ends of chains. It should be possible, then, to detect a change in quantum yield, or (in the case of estradiol which can fluoresce under certain conditions) a shift in λ_{max} of the emitted fluorescence if steroids bind to the ends of poly-7-methyl G chains. Equilibrium dialysis showed that only estradiol binds to the methylated polymer (Table IV). The fluorescence absorption and emission spectra were identical with those reported by Pochon *et al.* (1968) and were unchanged in the presence of up to 10^{-5} M estradiol, indicating that end binding of the steroid is unlikely. If it does occur, any quenching of fluorescence by the steroid or energy transfer to the steroid are not measurable by this method.

The lack of binding of progesterone and testosterone to

TABLE III: Binding Constants[a] and Numbers of Binding Sites[a] Derived from Binding Isotherm in Figure 4.

n1	2×10^{-5}
K1	6.0×10^6 (M^{-1})
n2	2×10^{-4}
K2	3.6×10^5 (M^{-1})

[a] These values are derived from a simplified resolution of the binding isotherm into two main components.

TABLE IV: Binding of Steroids to Poly G and Poly-7-methyl G in HMP Buffer.

Steroid	nK (M^{-1})	
	Poly G	Poly-7-methyl G
Progesterone	1100	0
Testosterone	221	0
Estradiol	280	82

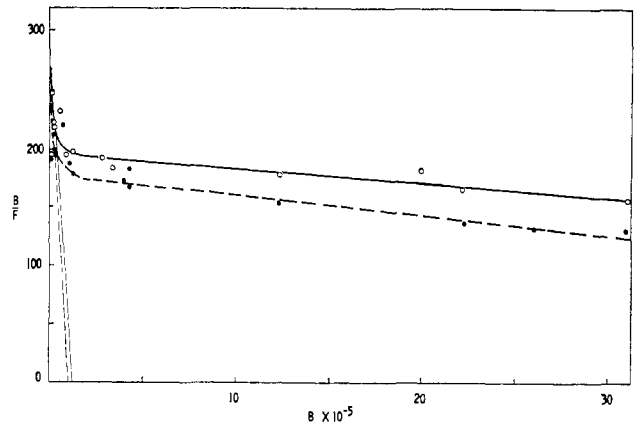

FIGURE 5: Scatchard plots of testosterone binding to alkali-denatured' sheared (—○—), and unsheared (—●—) *Ps. aeruginosa* DNA in HMP buffer. Equilibrium dialysis at 4° for 48 hr.

methylated poly G is of interest in itself. A positive charge is introduced by methylation at N-7; the only change in optical rotatory dispersion, due to introduction of the methyl group, observed in the present studies was a negative rotation of the Cotton peak at 228 mμ. It is possible that relatively small changes in stacking of guanine residues in poly-7-methyl G are sufficient to exclude binding of testosterone and progesterone but that the altered polymer structure to some extent still meets the less stringent requirements for estradiol binding. However, the altered purine electronic structure, *per se*, may be responsible in part for the altered binding properties.

Effects of DNA Secondary Structure on Binding of Steroids. Although binding of steroids to the ends of polynucleotide chains is now seen to be unlikely, there remains the perplexing observation that the number of steroid binding sites, on polynucleotides, corresponding to maximum binding constants is less than 1/10,000 nucleotide residues (Cohen *et al.*, 1969). In an attempt to interpret these observations, studies were carried out with *Ps. aeruginosa* DNA of varying size, denatured under differing conditions. In a series of initial experiments using different *Ps. aeruginosa* DNA preparations, considerable variation was observed in nK values for a given steroid binding to heat-denatured DNA; this was also true for a given steroid with aliquots of a single preparation of DNA denatured separately on different occasions. *Ps. aeruginosa* DNA has a high G + C content (67%) and these variable nK values undoubtedly reflect the variable extent of denaturation and renaturation, as evidenced by somewhat variable values for residual hyperchromicity after heating and rapid cooling. More reproducible data were obtained with alkali-denatured DNA: the results of a comparative experiment are shown in Table V. In general, nK values were found to be higher with alkali-denatured than with heat-denatured DNA. Shearing of the DNA by sonication increased the nK values in both cases, which could reflect either preferential binding to ends of chains or more complete denaturation of the shorter molecules. Binding isotherms with testosterone, for example, were similar for sheared and unsheared, alkali-denatured DNA (Figure 5), the number of binding sites corresponding to the maximal binding constant being of the order of 10^{-5}. The number of binding sites corresponding to the lesser slope of the binding isotherm was at least two orders of magnitude greater; saturation of these binding sites would occur only near the solubility limits of the steroid in aqueous buffer.

These observations on the binding of steroids to *Ps. aeruginosa* DNA suggest that the extent of binding is dependent on the extent of denaturation, *i.e.*, optimal binding sites can be limited in number. At the same time, where denaturation was the more satisfactory (alkali-denatured DNA), a 50-fold reduction in size of DNA did not greatly alter the binding properties of the polymer as judged from the binding isotherms, despite some differences in nK values at a single steroid concentration. Since a 50-fold increase in the number of the chain ends occurred in this case, these data further indicate that end binding of the steroids is unlikely.

General Comment

On the basis of these and previous experiments, it is possible to draw a number of conclusions about the role of polymer structure in associations of steroids with polynucleotides. First, there is a requirement for single-stranded regions (Cohen and Kidson, 1969b) in both ribo and deoxyribo series. These regions may be located either on linear or on

TABLE V: Relation of *Ps. aeruginosa* DNA[a] Structure to Steroid Binding in HMP Buffer.

Steroid	nK (M^{-1})			
	Heat-Denatured DNA		Alkali-Denatured DNA	
	Unsheared	Sheared	Unsheared	Sheared
Testosterone	28	58	149	203
Progesterone	67	145	305	416

[a] A single batch of native DNA in HMP buffer was sheared by sonication, then half of the batch of sheared DNA was denatured by heat, the other half by alkali. Sedimentation constants were determined in the analytical ultracentrifuge on the native DNA before and after shearing. For unsheared DNA, $s^0_{20,w} = 28.6$ S (18×10^6 mol wt) and for sheared DNA, $s^0_{20,w} = 7.1$ S (3.2×10^5 mol wt).

circular molecules in the case of DNA: in both instances, the influence of ionic strength suggests that base stacking is important for interactions with progesterone and testosterone but less so for interactions with estradiol. Similarly, with poly G, the extent of base stacking is important, as evidenced by the effects of protonation (Cohen *et al.*, 1969) or methylation of guanine residues at N-7 (Table V), although here the effects due to altered electronic structure of the purine may also play a role. The observations that increased stacking is favorable to the binding of progesterone and testosterone but less so to the binding of estradiol is compatible with association between each steroid and a short nucleotide sequence as demonstrated by model building (Cohen *et al.*, 1969). The idea that steroid associations are likely to occur along polynucleotide chains is supported by the present data excluding end binding: binding to circular DNA, binding to T_7 DNA which lacks terminal guanine residues, the similarity of binding to sheared and unsheared *Ps. aeruginosa* DNA, the absence of evidence of altered fluorescence of poly-7-methyl G in the presence of estradiol. In retrospect, additional support for this conclusion can be drawn from certain of the previous data: the absence of steroid binding to native DNA, which should contain a proportion of terminal guanine residues; the absence of binding to native poly dG:dC, which does contain terminal guanine residues; the absence of binding to poly (C,G), which should contain some available guanine termini, even though others may be masked by excess cytosine residues. In addition, the virtual absence of binding of desoxy derivatives of the three steroids (Cohen *et al.*, 1969) would be surprising if end binding due to hydrophobic interactions were the rule, since these derivatives are more hydrophobic than the parent steroids. Finally, except in the case of ring A of estradiol the nonplanar steroid structures do not favor associations with the planar surface of a terminal guanine residue. One alternative to binding of the steroid along a nucleotide sequence which is not excluded by the data is association with a G:G pair. However, it is not clear from model building how this could occur.

There remains the important question of the low number of optimal binding sites. The free energy of binding at these sites is of the order of 7–10 kcal/mole of steroid bound (Cohen *et al.*, 1969), which is rather high for two hydrogen bonds in water. It is conceivable, then, that optimal binding sites in single-stranded polynucleotides are provided by relatively hydrophobic, coiled regions where there is partial exclusion of water. Sites where the free energy of binding is less thus might reflect less protected regions of the polynucleotide where water molecules compete more favorably in the formation of hydrogen bonds with functional groups of both steroid and purine. It is possible, of course, that high energy binding sites reflect steroid associations with a contaminant such as protein, even though the protein content of the DNA and polynucleotide preparations used were below the level of detection. However, the homopolymer and copolymer specificity, and the absence of binding to native DNA (Cohen and Kidson, 1969b) make this unlikely. Binding to an unusual base in DNA or even in poly G or poly I cannot be excluded.

In a homopolymer such as poly G, the great majority of guanine residues are involved in hydrogen-bonded, multistrand structures (Pochon and Michelson, 1965), so that the low number of binding sites might largely reflect the availability of suitable single-stranded regions. In denatured DNA the occurrence of a certain amount of random base pairing will tend to restrict the number of binding sites available: The present data do indeed indicate that the number of optimal binding sites depends on the extent of denaturation (Table V). However, it is important to note that a much greater number of suboptimal binding sites exists (Figure 5) and it is possible that for a given steroid all binding sites are chemically identical but that the free energy of binding varies with the extent of exposure to water of different regions of the polymer. This certainly should be true in the coiled structures assumed by denatured DNA. Binding does not occur to random guanine residues and there is good reason to believe that a pentanucleotide of restricted sequence may be necessary to define a steroid binding site (Kidson *et al.*, 1970). Such requirements would further restrict the expected number of binding sites on a given polynucleotide. These conclusions can be tested by analysis of steroid binding to oligonucleotides or to particular regions of nucleic acids which are known to be more exposed to water.

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References

Cohen, P., Chin, R. C., and Kidson, C. (1969), *Biochemistry 8*, 3603.
Cohen, P., and Kidson, C. (1969a), *Fed. Proc. 28*, 531.
Cohen, P., and Kidson, C. (1969b), *Proc. Natl. Acad. Sci. U. S. 63*, 458.
Davison, P. F., and Freifelder, C. (1962), *J. Mol. Biol. 5*, 643.
Davison, P. F., Freifelder, C., and Holloway, B. W. (1964), *J. Mol. Biol. 8*, 1.
Kidson, C. (1966), *J. Mol. Biol. 14*, 1.
Kidson, C., Cohen, P., and Chin, R. C. (1970), *in* The Sex Steroids: Molecular Mechanisms, McKerns, K. W., Ed., New York, N. Y., Appleton-Century-Crofts (in press).
Lehman, I. R. (1960), *J. Biol. Chem. 235*, 1479.
Lehman, I. R. (1963), *Methods Enzymol. 6*, 40.
Leng, M., Pochon, F., and Michelson, A. M. (1968), *Biochim. Biophys. Acta 169*, 338.
Michelson, A. M., and Pochon, F. (1966), *Biochim. Biophys. Acta 114*, 469.
Parish, J. H., and Kirby, K. S. (1966), *Biochim. Biophys. Acta 129*, 554.
Pochon, F., Leng, M., and Michelson, A. M. (1968), *Biochim. Biophys. Acta 169*, 350.
Pochon, F., and Michelson, A. M. (1965), *Proc. Natl. Acad. Sci. U. S. 53*, 1425.
Richardson, C. C. (1966), *J. Mol. Biol. 15*, 49.
Scatchard, G. (1949), *Ann. N. Y. Acad. Sci. 51*, 660.
Sinsheimer, R. (1959a), *J. Mol. Biol. 1*, 37.
Sinsheimer, R. (1959b), *J. Mol. Biol. 1*, 43.
Studier, F. W. (1965), *J. Mol. Biol. 11*, 373.
Thomas, C. A., and Abelson, J. (1966), *in* Procedures Nucleic Acid Research, Cantoni, G. L., Davies, D. R., Ed., New York, N. Y., Harper & Row, p 553.
Weiss, B., and Richardson, C. C. (1967), *J. Mol. Biol. 23*, 405.