J. Steroid Biochem. Molec. Biol. Vol. 39, No. 2, pp. 133–146, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

STEREOCHEMICAL COMPLEMENTARITY OF PROGESTERONE AND CAVITIES BETWEEN BASE PAIRS IN PARTIALLY UNWOUND DOUBLE STRANDED DNA USING COMPUTER MODELING AND ENERGY CALCULATIONS TO DETERMINE DEGREE OF FIT

LAWRENCE B. HENDRY* and VIRENDRA B. MAHESH
Department of Physiology and Endocrinology CLW 3138, Medical College of Georgia, Augusta, GA 30912, U.S.A.

(Received 24 September 1990)

Summary—Computer modeling was applied for the first time to investigate previously reported complementarity of progesterone and cavities formed between base pairs in partially unwound double stranded DNA. Computer graphics enabled a more objective assessment of complementarity; energy calculations provided a rigorous method to evaluate degree of fit. Graphics confirmed that the complementarity was virtually "lock and key", i.e. close contacts were formed between van der Waals surfaces in the progesterone/DNA complexes and hydrogen bonds were formed between the two carbonyl groups on opposite ends of the steroid and phosphate groups on adjacent strands of DNA. Molecular mechanics calculations revealed that insertion of the steroid resulted in a relatively stable complex i.e. both van der Waals and electrostatic energies were lowered due to favorable steric interactions and stereospecific hydrogen bonds, respectively. Three published X-ray crystal structures of progesterone exhibited similar complementarity. Ent-progesterone which does not occur naturally possessed very poor complementarity. These findings confirm that the structure of progesterone is directly reflected in the stereochemistry of DNA. While no mechanistic explanation for these results is proffered, we hypothesize that such complementarity must have played a decisive role in the evolution of steroid hormone structure and function.

INTRODUCTION

Previous modeling studies in our laboratories demonstrated that a variety of small molecular weight, biologically active natural products display stereochemical complementarity with double stranded DNA [1–17]. Remarkable complementarity was exhibited by steroid hormones which were capable of inserting into cavities between base pairs in partially uncoiled DNA. Each steroid formed stereospecific hydrogen bonds linking phosphate groups on adjacent DNA strands. For example, the female reproductive hormone progesterone formed linkages involving both the 3 and the 20 carbonyl oxygens.

Much of the early investigations of steroid/DNA complexes were conducted with physical models i.e. Corey–Pauling–Koltun (CPK) space-filling models followed by Kendrew skeletal models and then silastic polymer models constructed from computer derived X-ray coordinates [1–3]. Herein, we report for the first time the application of computer graphics and energy calculations to this problem using progesterone as the candidate steroid. The results support earlier descriptions and demonstrate that progesterone is a stereochemical "lock and key" fit into partially unwound DNA.

Before proceeding to the body of this paper, we would like to emphasize to the reader that the primary rationale for these studies has **not** been to suggest that steroid hormones in concert with receptor proteins interact directly with DNA during their mode of action or for that matter to provide a framework to study physicochemical interactions of steroids and nucleic acids. The main impetus for these studies has been the search for an answer to the question of why the structures of steroids and other biologically active natural products exist. Given that the genes ultimately contain this information, our hypothesis has been that this structural information is manifest directly in the stereochemistry of nucleic acids. If this hypothesis is correct, the discovery of stereochemical complementarity between steroid hormones and DNA

*To whom correspondence should be addressed.

is undoubtedly a reflection of relationships that took place at a crucial stage in the evolution of the steroid structure prior to the development of receptor proteins and other regulatory factors involved in hormone action.

EXPERIMENTAL

Molecular modeling was conducted on a Digital Microvax II computer interfaced via Ethernet to an Evans and Sutherland PS390 graphics computer equipped with a stereographics viewer. The software employed was Sybyl/Mendyl 5.3 (Tripos Associates, St Louis, MO). The Cambridge X-ray crystallographic database [18] installed on the Microvax was used to access the structures of small molecules. The Sybyl/Mendyl force field was employed in all energy calculations; the van der Waals parameter for hydrogen atoms was altered from 1.5 to 1.2 Å to more accurately reflect potential steric interactions.

Double stranded dinucleotides representing partially unwound DNA were constructed from crystallographically derived fragment libraries in the Biopolymer program. The sequence employed herein was 5′-dTdG-3′.5′-dCdA-3′ which was previously reported to accommodate steroid hormones [1–3]. The deoxyribose conformation for each of the sugars was 3′-endo similar to that reported for the intercalation of proflavine into DNA [19–21]. In order to form the cavity, the DNA was unwound by twisting the 14 possible torsional angles on the backbone. In this manner, the base pairs were separated by a distance comparable to the approximate width of a steroid. During this procedure, the established distances of the hydrogen bonds between the base pairs were maintained using monitor pairs. Kollman charges were then generated for the final DNA conformation.

The progesterone structures were obtained from the Cambridge database [18] and are also available in the *Atlas of Steroid Structure* [22, 23]; the primary references are as follows: progesterone No. 1 [24], progesterone No. 2 [25], progesterone No. 3 [26]. Ent-progesterone was generated by inverting the chirality of progesterone No. 1. Charges for each structure were generated using the Tripos combined Gasteiger–Huckel method which includes both σ and π bonding contributions in the calculation.

To facilitate docking of the steroids into the cavity in DNA, van der Waals dot surfaces were generated. Each steroid was then inserted into the cavity using the stereoviewer without permitting overlap of the surfaces. The distances between each carbonyl group on the steroid and phosphate oxygens on adjacent DNA strands were also monitored interactively in order to optimize the direction and distances of potential hydrogen bonds.

The relative fit of each steroid into DNA was calculated using the force field by assessing the optimum favorable change in energy resulting from docking the ligand. A convenient technique was to define the steroid and DNA separately as aggregates, perform the docking procedure, merge the structures and then calculate: the steric fit in the complex from the van der Waals energy; the hydrogen bonding fit from the electrostatic interaction of potential donor/acceptor heteroatoms. Because the Sybyl/Mendyl force field does not specifically contain a hydrogen bonding term, the latter calculation considered only the electrostatic interaction of the carbonyl oxygens and protons attached to the phosphate groups. In order to compare the relative fit of each steroid in the cavity, the values in kcal calculated for the van der Waals energy were added to those for the hydrogen bonding energy and then normalized to the best fitting structure. It should be noted that while the energies reported here were calculated by accepted methods in computational chemistry that are based upon established physicochemical principles [27], they were not empirically derived. Thus, the absolute values in kcal have little independent experimental significance but are valuable indicators of relative degree of fit of candidate structures.

RESULTS

Computer modeling demonstrated that progesterone was capable of inserting fully between base pairs in the partially unwound cavity in DNA. Fit within the steroid/DNA complex was manifest in complementary steric contacts as well as stereospecific hydrogen bonds between the two carbonyl groups on the steroid and protonated phosphate oxygens on adjacent DNA strands. Each of the three available X-ray crystal structures of progesterone fit into DNA in a similar fashion.

As exemplified using progesterone No. 1, the van der Waals surfaces of the basic steroid nucleus depicted by both dot surfaces and space-filling images filled the space between base

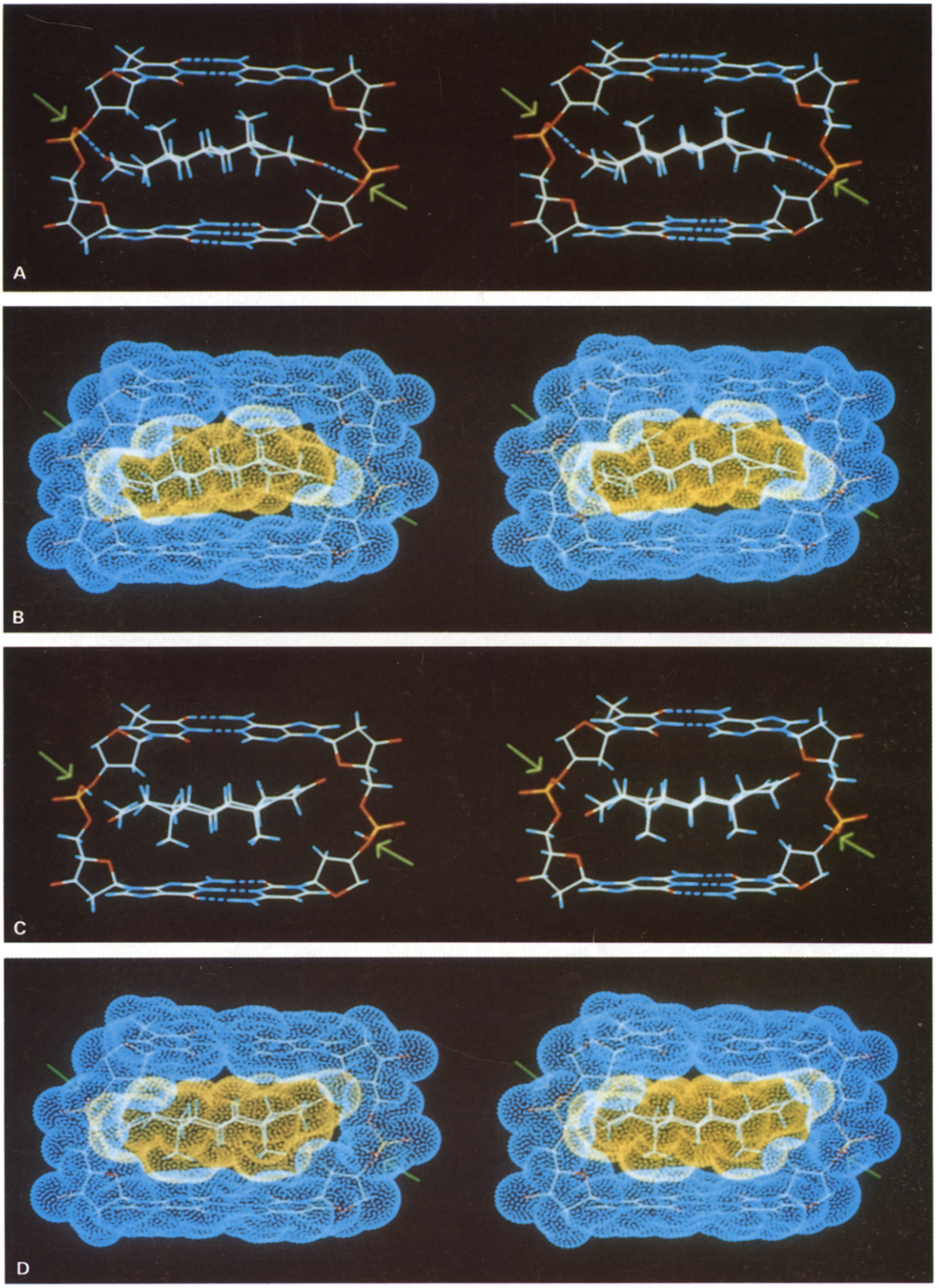

Fig. 1. Computer modeling depicting the fit of progesterone (X-ray crystal structure No. 1) in the partially unwound DNA cavity, 5′-dTdG-3′.5′-dCdA-3′ using skeletal models (A and C) and dot surfaces (B and C). The latter represent van der Waals surfaces. Stereo images are shown with all views from the major groove. Dashed blue lines indicating hydrogen bonds; green arrows indicate the position of phosphate groups which can act as proton donors. (A, B) Progesterone inserted into DNA. (C, D) Attempt to insert ent-progesterone into DNA; note that the direction of the carbonyl groups (red) in ent-progesterone precludes formation of suitable complementary hydrogen bonds to DNA.

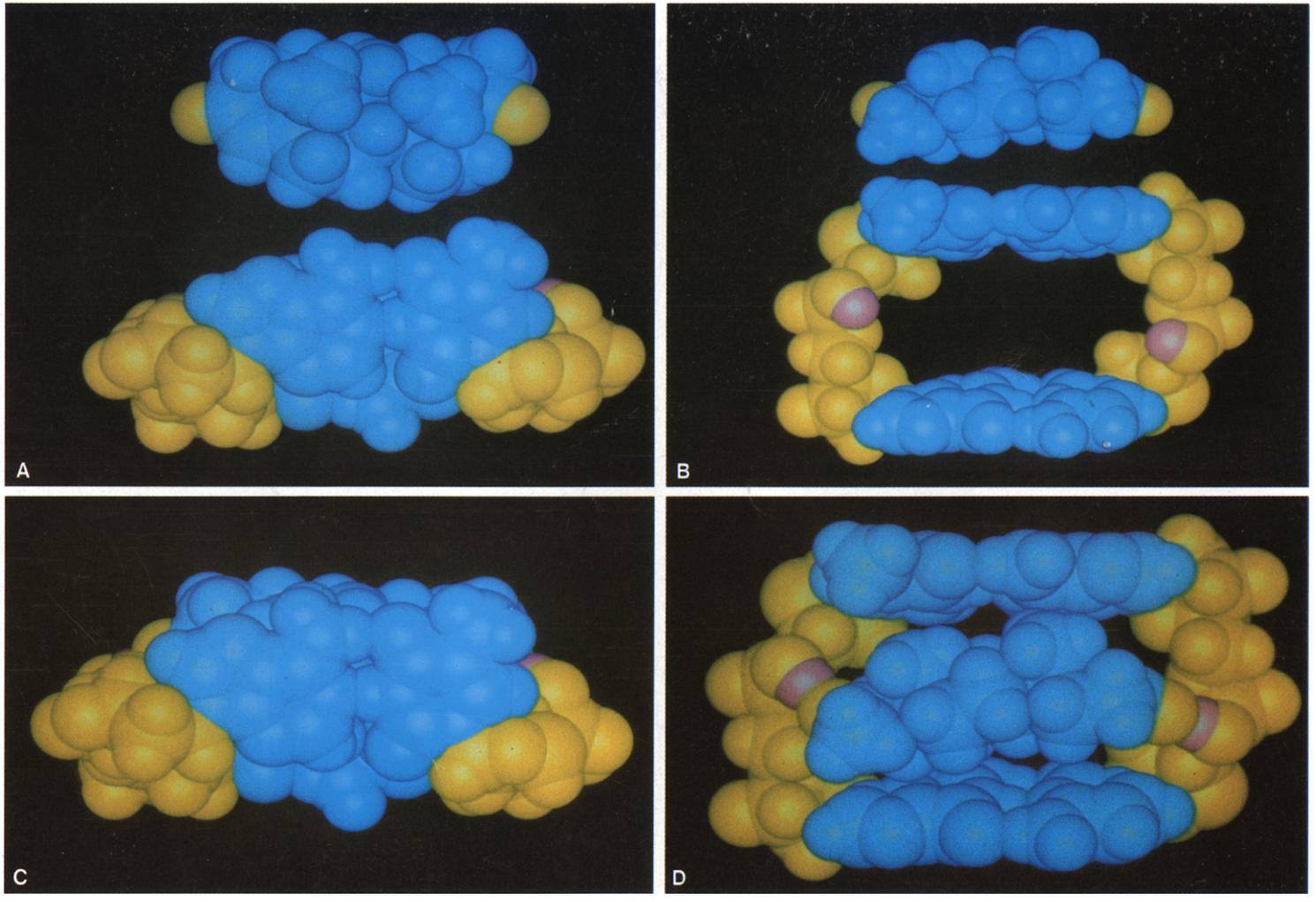

Fig. 2(A–D)—*legend on p. 140.*

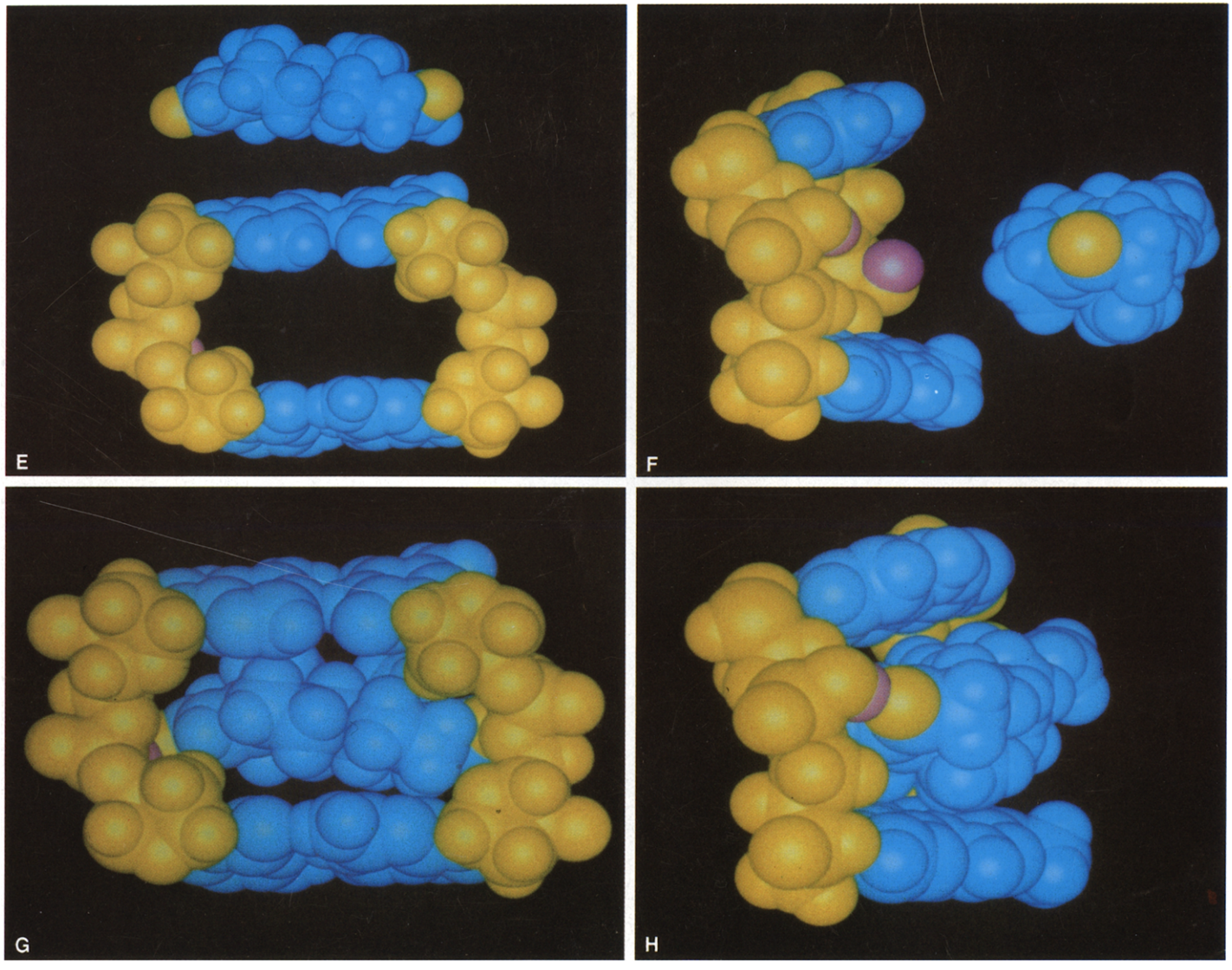

Fig. 2(E–H)—*legend overleaf.*

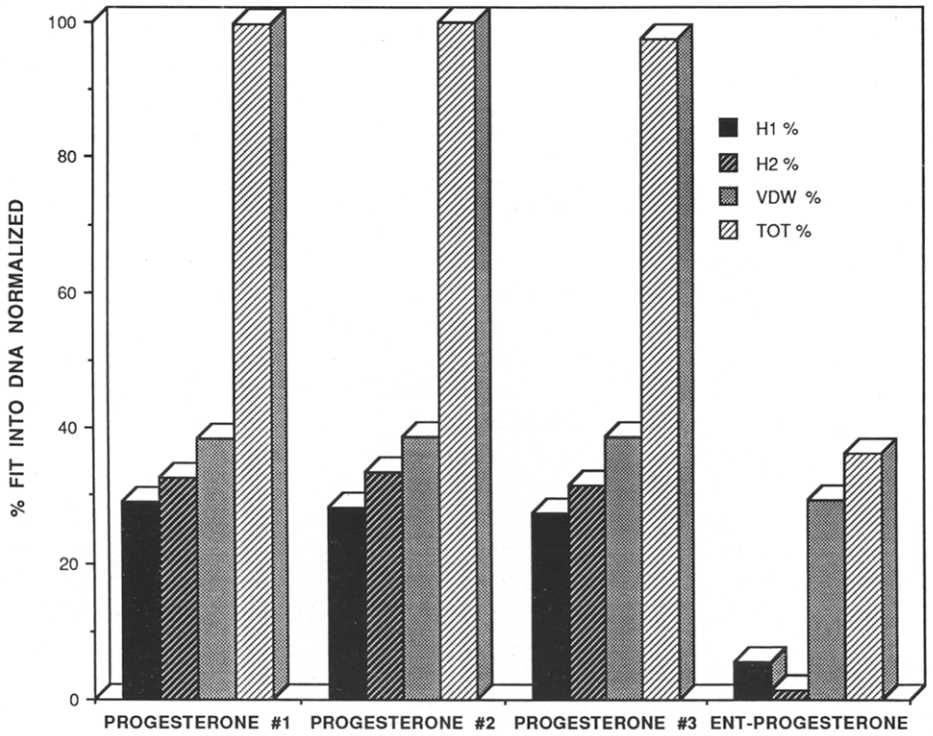

Fig. 3. Fit of various progesterone X-ray crystal structures into DNA measured by force field energy calculations (see text for discussion). The bars represent the relative favorable energy change resulting from insertion of the steroid. The electrostatic energies for hydrogen bonds to the phosphate groups of each strand are designated H1%(5′-dCdA'3′) and H2% (5′-dTdG-3′) with the van der Waals energy shown as VDW%. The total energy change is designated TOT%.

Fig. 2(A–H)—*see pp. 138–139.*

Fig. 2. Computer generated space-filling models of the complex in Fig. 1 illustrating the "lock and key" complementarity of progesterone and DNA (see text for discussion). Hydrophobic and hydrophilic regions of each molecule are colored blue and yellow, respectively. The two phosphate protons which form hydrogen bonds are colored magenta. Various perspectives of progesterone and the DNA cavity prior to insertion are A, B, E and F with the respective complexes shown from the same perspective below. (A, C) The β face of the steroid is shown with the two carbonyl oxygens colored yellow and the cyclopentanophenanthrene skeleton colored blue; the DNA is viewed down the helix axis from above the blue AT base pair bordered by the yellow deoxyribose–phosphate backbone. The wider major groove is on the upper portion of the photograph with the minor groove situated below. (B, D) The view of the steroid is in the plane of the skeleton with the thicker side showing (carbons 2, 1, 11, 12, and 21); the DNA is viewed from the major groove with the same perspective as Fig. 1. (E, G) The view of the steroid is in the plane of the skeleton with the thinner edge showing (carbons 4, 6, 7, 15, 16); the DNA is viewed from the relatively narrow minor groove. (F, H) The steroid is shown on end from the side of the A ring and 3 carbonyl group (note the wedge shape); the view of the DNA is along the sugar–phosphate backbone 5′-dCdA-3′ with the major groove on the right and the minor groove to the left.

(2) the electrostatic interactions between the carbonyl groups of progesterone and phosphate protons indicated good hydrogen bonding interactions (i.e. −12–14 kcal each); (3) each of the reported X-ray crystal structures of progesterone gave similar results; and (4) ent-progesterone was a relatively poor fit (35%) in comparison to the natural isomer.

In previous modeling studies, there were inherent limitations in assessing complementarity of steroids and DNA. In addition to the possibility of introducing investigator bias due to the relatively primitive nature of the physical models employed, we were unable to rigorously quantitate the degree or absence of fit. While this initial computer modeling study eliminates these difficulties and is far superior to previous methods, we would like to point out some of the current limitations of this initial study and how they might be addressed. In the construction of the DNA, a single sugar conformation, 3′-*endo*, was employed. This conformation was chosen because of its prevalence in intercalation complexes e.g. proflavine and DNA [19–21]. In the future, DNA constructed with different combinations of deoxyribose conformations will be studied in a systematic fashion. To date, however, results obtained by fitting progesterone into DNA cavities constructed with mixed sugar puckers have been similar to those reported here. Interestingly, studies in progress indicate that different steroid hormones are accommodated by different conformations of DNA resulting primarily from changes in the torsional angles on the sugar phosphate backbone. For example, estradiol, which is smaller than progesterone in width and length due to the aromatization of the A ring and lack of a side chain at C17, respectively, fits into a more compact cavity derived from less unwinding of the helix. The energetics of fit of estradiol (total approx. −60 kcal) are comparable to progesterone (total approx. −45 kcal). As reported with space filling models, the larger biosynthetic precursors of progesterone are relatively poor fits into DNA [1]. Current data derived from computer graphics and energy calculations confirm the poor fit of these precursors (e.g. lanosterol lowers the energy only a total of approx. −16 kcal). Remarkably, there also appears to be a progressively increased fit of the intermediates along the biosynthetic pathway. Details of these studies will be the subject of subsequent publications.

Only one double stranded DNA dinucleotide sequence was thoroughly examined in this study. However, given that the deoxyribose–phosphate backbone is common to all DNA sequences, unwinding the helix results in cavities in which the phosphate groups are in the same relative position at the border of all possible cavities. Thus, insertion of progesterone into any cavity can result in the same stereospecific hydrogen bonds between progesterone and the DNA backbone regardless of sequence from which the cavity was derived. Conversely, for this reason, no sequence is capable of accommodating ent-progesterone. Work in progress involves insertion of progesterone into cavities formed from other sequences including two different sequences in which the GC base pair was replaced by AT (5′-dTdA-3′.5′-dTdA-3′) and the TA base pair was replaced with GC (5′-dGdG-3′.5′-dCdC-3′). Progesterone can form complexes with these sequences in which the hydrogen bonding distances and respective electrostatic energies are within the range reported above for the progesterone/5′-dTdG-3′.5′-dCdA-3′ complex. Upon insertion of the steroid, the van der Waals energies in these complexes are lowered 0.2–0.3 kcal less which reflects less contact of the progesterone molecule with the surfaces of the base pairs. While the trend of these results are consistent with those reported previously with space filling and silastic polymer models [1], we must caution that in the current computer investigations it was not possible to impose limitations on the borders of the complexes in the major and minor grooves. Such borders previously depicted in silastic polymer models are consistent with positions of water molecules surrounding the complexes and suggest sequence specificity of the fit of progesterone and other steroid hormones into DNA. In this initial study, no conclusions about sequence specificity should be drawn from the computer calculations until it is possible to include solvent shells, analyses of cavities derived from all possible sequences and molecular dynamics analyses of these complexes.

For accuracy, X-ray crystallographic coordinates for progesterone were employed in this study. We have also constructed progesterone and other steroids from fragment libraries and subjected the structures to energy minimization using the Sybyl/Mendyl force field. While this method generally resulted in a flatter steroid skeleton, the docking studies gave results

comparable to those reported above. In the future, studies will also be conducted with longer DNA sequences; this will enable assessment of the effect that insertion of the steroid has on the conformations of neighboring base pairs. To date, it has been possible to construct partially unwound cavities from the double stranded B form of DNA which are embedded within a larger sequence as well as to insert progesterone, however, the computational requirements to calculate the effects on the entire sequence are beyond the scope of our current methodology. Space filling models have clearly indicated that due to the location of the phosphate groups it is not possible to fit progesterone into partially unwound Z-DNA and maintain stereospecific hydrogen bonding [1]. We have obtained some intriguing preliminary results with space filling models of cruciform structures of DNA arising from palindromic sites containing double stranded 5′-dTdG-3′.5′-dCdA-3′ within the loops. These sites can be unwound facily and appear to be particularly accessible to insertion by the steroid.

It is also clear that a variety of environmental factors will effect the fit of steroids in DNA such as pH, metal cations, ionic strength as well as the type and degree of solvation. As discussed above, it is our intention to eventually investigate progesterone/DNA complexes which include solvent shells of water [28]. Water molecules can also act as hydrogen bonding spacers between phosphate and carbonyl groups. Similarly, the divalent ions calcium and magnesium can serve as stereospecific bridges between phosphate oxygens and carbonyl groups in a manner similar to the hydrogen bonds described above. The effects of chromosomal proteins on such complexes can also be envisioned. For example, the ability of phosphate groups to serve as proton donors or acceptors could be precisely determined by the nature of the amino acid residues of a protein in close proximity to the site. In addition to regulating the electrostatic properties of the DNA, such proteins could provide additional specificity by recognizing which sequences would be available for insertion of the steroid as well as regulating the degree of unwinding of the DNA necessary to accommodate a particular ligand. This scenario would potentially allow for greater specificity in the interaction of different classes of steroid hormones with cavities in DNA.

It cannot be overemphasized that these findings further substantiate that there is a conformation of a double stranded dinucleotide which is capable of accommodating progesterone. As stated in the Introduction, it should not be inferred, nor is it our hypothesis, that the mode of action of progesterone which is mediated by receptor proteins involves a stage in which the steroid is inserted into DNA. This question cannot be addressed by computer modeling alone, moreover, there is extensive evidence in the literature that steroids bind very weakly to DNA [29–38]. Studies in our laboratories using various DNA preparations and synthetic polydeoxynucleotides further support this contention [2, 5]. We found that, regardless of the experimental approach (DNA melting, fluorescence, equilibrium dialysis), only weak steroid/DNA binding was measurable. This was the case even with the relatively flat phytoestrogen, coumestrol. Thus, in the absence of data demonstrating strong binding of steroids and DNA coupled with our relative lack of knowledge of the details of the complex interactions of the receptor proteins with DNA *in vivo*, no mechanistic interpretations can be seriously considered. This is not the case with classical intercalating agents whose mode of action is known to involve direct interaction with DNA [19]. In contrast to the steroids, classical intercalators are generally flat aromatic structures which bind strongly to DNA upon insertion between base pairs. Intercalators also readily lend themselves to spectroscopic investigations which can provide accurate measurements of the degree of unwinding of the helix. For example, Kollman has successfully coupled NMR data with computer modeling to investigate the intercalation of actinomycin into DNA [39]. In that study, it was also possible to assess the energy required to unwind the helix. Interestingly, classical intercalators, however, by virtue of their flatness generally possess very poor topographical complementarity with the cavities between base pairs particularly when compared to wedge shaped steroid hormones which are almost perfect complements of the space between the base pairs.

We recognize that the discovery of the fit of progesterone and other steroid hormones into DNA raises many questions. Is it possible that the complementarity described is fortuitous? Could the molecules have been forced to fit together? While it is not possible to remove all potential bias with the current modeling, all of

our studies have consistently shown that regardless of the method chosen progesterone is a "lock and key" fit into DNA. In our view, the precise location of the carbonyl groups which facilitate stereospecific hydrogen bonding with the DNA coupled with the close match of the shape of the steroid and that of the DNA cavity cannot be coincidental. The improved accuracy of the current method shows not only that progesterone fits into DNA but also permits quantitative measurement of the degree of fit. The finding that insertion of progesterone into the cavity results in a consistent and significant lowering of energy based on force field calculations indicates a relatively stable complex. Moreover, several different X-ray crystal structures were used and both van der Waals and hydrogen bonding energies were favorable within the complex. It is of further interest that the hydrophobic and hydrophilic portions of the progesterone structure complement those of the cavity in DNA, i.e. the carbonyl groups align with the external hydrophilic backbone whereas the hydrophobic steroid skeleton is stacked between the internal hydrophobic space between the base pairs. In fact, a persuasive argument can be made that the steroid structure predicts many aspects of the DNA structure as well as vice versa. Although not shown, a large majority of small molecules do not fit into DNA with the notable exceptions being hormonal structures [1, and references therein]. For example, we have reported using space filling models that many structural alterations in the basic steroid nucleus result in molecules which do not fit into DNA; similarly, deviations from the locations of heteroatoms on steroid hormones generally do not permit stereospecific hydrogen bonding to DNA [3]. With regard to the latter, when all possible positions on the 4-pregnene, estratriene and 4-androstene skeletons are substituted with either hydroxyl or carbonyl groups, only 8 of the more than 100 possible positions permit hydrogen bonding to phosphate groups. Each of the steroid hormones have functional groups in these locations; moreover, removal of one or more of these groups dramatically reduces biological activity.

The primary goal of our research has been the search for a rationale for the structures of biologically active natural products [17]. Other scientists have wondered whether such a rationale exists. For example, Bloch [40] stated: "The question is rarely asked, why is it that only certain chemical structures and not others are present in living systems? Or, put differently, can we rationalize the detailed design and architecture of molecules that evolution has selected from an essentially unlimited organic reservoir?" The central hypothesis underlying the current report as well as our previous studies has been that the structures of biologically active naturally occurring small molecules are reflected in the structure of DNA. With regard to steroid hormones, several investigators [41–44] including Huggins and Yang in 1962 [45] have remarked about the enigmatic, yet striking, close structural relationships between base pairs and steroids. Our discovery that the steroid structure is a complement of cavities between base pairs in DNA provides an explanation for these relationships. It is also reasonable to speculate that the unique cyclopentanophenanthrene nucleus which has evolved as an important structural motif for hormones was a direct consequence of such relationships.

Steroids are ubiquitous in nature and are widely distributed in both the plant and animal kingdoms. Even some very primitive organisms require steroids for growth such as mollicutes which are wall-less procaryotes [46, 47]; biosynthesis of sterols also occurs in several species of amoebae [48, 49]. While steroids have been identified as pheromones in a primitive water mold [50], hormonal functions for molecules with steroid structures did not develop in plants. In fact, hormonal steroids arrived relatively late in evolution [51, 52]. Hormonal function for steroids was eventually developed in insects where ecdysone, a steroid which causes chromosomal puffs, is known to regulate molting [53, 54]. Relatively few steroids out of a myriad of possibilities have evolved as hormones in higher animals. Certainly, the evolution of steroidogenic enzymes coupled with the later appearance of a closely related family of receptor proteins which guided the function of steroid hormones in higher animals provided two critical constraints on the structures of the hormones. Exactly how each step in the mechanism of action of steroid hormones developed leading to the regulation of genes is unknown but widely discussed [40, 51, 55–65]. While gene regulation is well accepted as a primary action of steroid hormones, it should also be mentioned that there is an increasing interest in extragenomic actions of steroids and in particular rapid membrane effects [66–68]. For

example, steroids can effect the mammalian central nervous system by binding to γ-aminobutyric acid receptors [69–74]. Similarly, release of LHRH mediated by progesterone has been proposed to occur through a membrane mechanism [75]. In any case, our findings that the steroid skeleton closely matches spaces between base pairs and that those steroids with hormonal function have hydrophilic groups uniquely positioned on the steroid framework to form complementary hydrogen bonding with hydrophilic groups on DNA [3] indicates that nucleic acids were intimately involved at various evolutionary stages eventually leading to the selection of steroid structures for hormonal function.

The concept of complementarity in biological systems is of course not new and was described a half century ago by Pauling and Delbruck [76]. While the report was concerned primarily with complementarity in the enzymatic synthesis of molecules, a general argument was put forth. "Attractive forces between molecules vary inversely with a power of the distance, and maximum stability of a complex is achieved by bringing the molecules as close together as possible, in such a way that positively charged groups are brought near to negatively charged groups, electric dipoles are brought into suitable mutual orientations, etc. The minimum distances of approach of atoms are determined by their repulsive potentials, which may be expressed in terms of van der Waals radii; in order to achieve the maximum stability, the two molecules must have complementary surfaces, like die and coin, and also a complementary distributions of active groups." More than a decade later, this description of complementarity was exemplified in the discovery of the structure of DNA by Watson and Crick [77], i.e. the complementary hydrogen bonding patterns exhibited by the base pairs. Watson and Crick [77] also recognized the biological importance of complementarity in their famous quote "It has not escaped our notice that the specific pairing suggests a possible mechanism for the genetic material." Although our observations of stereochemical complementarity are manifest in the insertion of hormonal steroids and other biologically active small molecules into cavities in nucleic acids, they are entirely consistent with the general physicochemical principles outlined by Pauling and Delbruck and later demonstrated by Watson and Crick. It is our view that such complementarity was a primary force in nature's choice of biologically active molecules and that the general concept of complementarity will take on increasing importance in our understanding of the evolution of biological structure and function.

Acknowledgements—Funding for these studies was provided in part by the World Health Organization (Project No. 87005) and the National Institutes of Health (Grant No. DK 32046 from NIADDK, NIH to VBM). We also wish to acknowledge Dr Thomas Muldoon who collaborated with us on this project up until his untimely death in the spring of 1989.

REFERENCES

1. Hendry L. B.: Stereochemical complementarity of DNA and steroid agonists and antagonists. *J. Steroid Biochem.* **31** (1988) 493–523.
2. Lehner A. F., Muldoon T. G., Mahesh V. B., Bransome E. D. Jr. and Hendry L. B.: Initial Studies of a phytoestrogen–deoxyribonucleic acid interaction. *Molec Endocr.* **1** (1987) 377–387.
3. Hendry L. B., Bransome E. D. Jr., Lehner A. F., Muldoon T. G., Hutson M. S. and Mahesh V. B.: The stereochemical complementarity of DNA and reproductive steroid hormones correlates with biological activity. *J. Steroid Biochem.* **24** (1986) 843–852.
4. Hendry L. B., Muldoon T. G., Bransom E. D. Jr., Lehner A. F., Hutson M. S., Uberoi N. K., Myers R. B., Segaloff A. and Mahesh V. B.: Predicting hormonal activity from the stereochemistry of nucleic acids: a prospective study with novel estrogenic steroids. In *Advances in Gene Technology: Molecular Biology of the Endocrine System* (Edited by D. Puett, F. Ahmad, S. Black, D. M. Lopez, M. H. Melner, W. A. Scott and W. J. Whelan). Cambridge University Press, New York (1986) pp. 292–293.
5. Lehner A. F., Muldoon T. G., Mahesh V. B., Bransome E. D. Jr. and Hendry L. B.: The phytoestrogen coumestrol appears capable of insertion between base pairs in agreement with a stereochemical rationale for hormone action. In *Advances in Gene Technology Molecular Biology of the Endocrine System* (Edited by D. Puett, F. Ahmad, S. Black, D. M. Lopez, M. H. Melner, W. A. Scott and W. J. Whelan). Cambridge University Press, New York (1986) pp. 303–304.
6. Tyler J. M., Lehner A. F., Bransome E. D. Jr. and Hendry L. B.: Stereochemical complementarity of glucose with a specific site in DNA may be used to predict the structures and activity of drugs which modulate sugar metabolism. *Diabetes* **35** (1986) 138A.
7. Uberoi N. K., Hendry L. B., Muldoon T. G., Myers R. B., Segaloff A., Bransome E. D. and Mahesh V. B.: Structure-activity relationships of some unique estrogens related to estradiol are predicted by fit into DNA. *Steroids* **45** (1985) 325–340.
8. Bransome E. D. Jr., Hendry L. B., Muldoon T. G., Mahesh V. B., Hutson M. S. and Campbell L. K.: Apparent stereochemical complementarity of estrogens and helical cavities between DNA base pairs: implications for the mechanism of action of steroids. *J. Theor. Biol.* **112** (1985) 97–108.
9. Hendry L. B., Bransome E. D. Jr., Hutson M. S. and Campbell L. K.: Method of predicting biological activity of compounds by nucleic acid models. *U.S. Patent* **4,461,619** (1984)
10. Smith T. J., Hendry L. B. and Bransome E. D. Jr.: Are the stereochemistry and mechanism of action of thyroid hormones predicted by the structure of DNA? *Perspect. Biol. Med.* **27** (1984) 408–416.

11. Hendry L. B., Bransome E. D. Jr., Hutson M. S. and Campbell L. K.: A newly discovered stereochemical logic in the structure of DNA suggests that the genetic code is inevitable. *Perspect. Biol. Med.* **27** (1984) 623–651.
12. Kline E. L., Hendry L. B., Bransome E. D. Jr. and Chernin M. I.: Transcription initiation at pregene sequences in *E. Coli* with responses to small molecules (cAMP and indoles): evidence for stereospecificity in metabolite gene regulation. In *Promoters: Structure and Function* (Edited by R. L. Rodriquez and M. J. Chamberlin). Praeger, New York (1982) pp. 195–215.
13. Kline E. L., Hendry L. B., Bransome E. D. Jr. and Montefiori D. C.: Metabolite gene regulation (MGR): stereospecific induction of macromolecular synthesis by small molecules in both procaryotic and eucaryotic systems. In *Recombinant DNA* (Edited by A. G. Walton) Elsevier, New York (1981) pp. 229–243.
14. Hendry L. B., Bransome E. D. Jr., Hutson M. S. and Campbell L. K.: First approximation of a stereochemical rationale for the genetic code based on the topography and physicochemical properties of "cavities" constructed from models of DNA. *Proc. Natn. Acad. Sci. U.S.A.* **78** (1981) 7440–7444.
15. Hendry L. B., Bransome E. D., Muldoon T. G., Mahesh V. B., Campbell L. K. and Hutson M. S.: Unique stereochemical complementarity between estrogens and DNA. In *Functional Correlates of Hormone Receptors in Reproduction* (Edited by V. B. Mahesh, T. G. Muldoon, B. B. Saxena and W. A. Sadler). Elsevier, New York (1980) pp. 565–571.
16. Witham F. H., Hendry L. B. and Chapman O. L.: Chirality and stereochemical recognition in DNA–phytohormone interactions: a model approach. *Orig. Life* **9** (1978) 7–15.
17. Hendry L. B., Witham F. H. and Chapman O. L.: Gene regulation: the involvement of stereochemical recognition in DNA-small molecule interactions. *Perspect. Biol. Med.* **21** (1977) 120–130.
18. Cambridge Crystallographic Database, University Chemical Lab, Lenfield Rd, Cambridge, England.
19. Saenger W.: *Principles of Nucleic Acid Structure.* Springer, New York (1984).
20. Shieh H. S., Berman H. M., Dabrow M. and Neidle S.: The structure of drug–deoxydinucleoside phosphate complex. Generalized conformational behavior of intercalation complexes with RNA and DNA fragments. *Nucleic Acids Res.* **8** (1980) 85–97.
21. Berman H. M., Stallings W., Carrell H. L., Glusker J. P., Neidle S., Taylor G. and Achari A.: Molecular and crystal structure of an intercalation complex: proflavine-cytidylyl-(3′,5′)-guanosine. *Biopolymers* **18** (1979) 2405–2439.
22. Duax W. L. and Norton D. A.: *Atlas of Steroid Structure.* Plenum Press, New York, Vol. 1 (1975).
23. Griffin J. F., Duax W. L. and Weeks C. M.: *Atlas of Steroid Structure.* Plenum Press, New York, Vol. 2 (1984).
24. Campsteyn H., Dupont L. and Dideberg O.: Preg-4-ene-3,20-dione (progesterone). *Cryst. Struct. Commun.* **1** (1972) 219–222.
25. Serantoni E. F., Krajewski A., Mongiorgi R., Riva di Sanseverino L. and Cameroni R.: 4-Pregnen-3,20-dione (progesterone, form II). *Cryst. Struct. Commun.* **4** (1975) 189–192.
26. Dideberg O., Dupont L. and Campsteyn H.: Crystal and molecular structure of the 1:1 progesterone–resorcinol complex. *Acta Cryst.* **B31** (1975) 637–640.
27. Kollman P.: Theory of complex molecular interactions: computer graphics, distance geometry, molecular mechanism and quantum mechanics. *Acc. Chem. Res.* **18** (1985) 105–111.
28. Kopka M. L., Fratini A. V., Drew H. R. and Dickerson R. E.: Ordered water structure around a B-DNA dodecamer. *J. Molec. Biol.* **163** (1983) 129–146.
29. Munck A.: The interfacial activity of steroid hormones and synthetic estrogens. *Biochim. Biophys. Acta* **24** (1957) 507–514.
30. Munck A., Scott J. R. and Engel L. L.: The interaction of steroid hormones and coenzyme components. *Biochim. Biophys. Acta* **26** (1957) 397–407.
31. Ts'o P. O. P. and Lu P.: Interactions of nucleic acids, I. Physical binding of thymine, adenine, steroids and aromatic hydrocarbons to nucleic acids. *Proc. Natn. Acad. Sci. U.S.A.* (1964) 17–24.
32. Goldberg M. L. and Atchley W. A.: The effect of hormones on DNA. *Proc. Natn. Acad. Sci. U.S.A.* **55** (1966) 989–996.
33. Speichinger J. P. and Barker K. L.: Competitive effects of estradiol-17α on the estradiol-17β induced physical changes in uterine DNA. *Steroids* **14** (1969) 132–143.
34. Cohen P., Chin R. and Kidson C.: Interactions of hormonal steroids with nucleic acids. II. Structural and thermodynamic aspects of binding. *Biochemistry* **8** (1969) 3603–3609.
35. Cohen P. and Kidson C.: Interactions of hormonal steroids with nucleic acids, I. A specific requirement for guanine. *Proc. Natn. Acad. Sci. U.S.A.* **63** (1969) 458–464.
36. Kidson C., Thomas A. and Cohen P.: Interactions of hormonal steroids with nucleic acids III. Role of polymer structure. *Biochemistry* **9** (1970) 1571–1576.
37. Arya S. K. and Yang J. T.: Interaction of steroids with nucleic acids. *Biochemistry* **14** (1975) 963–969.
38. Blackburn G. M., Kellard B., Rashid A. and Thompson M. H.: Physical association of oestrogens and other steroids with DNA. *Biochem. Pharmac.* **32** (1983) 2571–2575.
39. Lybrand T. P., Brown S. C., Creighton S., Shafer R. H. and Kollman P. A.: Computer modeling of actinomycin D interactions with double-helical DNA. *J. Molec Biol.* **191** (1986) 495–507.
40. Bloch K. E.: Speculations on the evolution of sterol structure and function. *CRC Crit. Rev. Biochem.* **7** (1979) 1–5.
41, Dannenberg H.: Mode of action of carcinogenic factors. *Dt. Med. Wschr.* **88** (1963) 605–616.
42. Hoffmann S. and Witkowski W.: Intercalation. In *Theoretische und experimentelle Fortschritte der modifizierten Wirkstoffsuche* (Edited by H. Possin and G. Barysch). Martin-Luther-Universitat, Halle, Wittenberg (1980) pp. 29–53.
43. Sluyser M.: Interaction of steroid hormone receptors with DNA. *Trends Biochem. Sci.* **7** (1983) 236–238.
44. Sluyser M.: The role of the steroid molecule in hormonal action. In *Interaction of Steroid Hormone Receptors with DNA* (Edited by M. Sluyser). Ellis Horwood, England (1985) pp. 57–82.
45. Huggins C. and Yang N. C.: Induction and extinction of mammary cancer. *Science* **137** (1962) 257–262.
46. Rose D. L., Kocka J. P., Somerson N. L., Tully J. G., Whitcomb R. F., Carle P., Bové J. M., Colflesh D. E. and Williamson D. L.: *Mycoplasma lactucae* sp. nov., a sterol-requiring mollicute from a plant surface. *Int. J. Syst. Bact.* **40** (1990) 138–142.
47. Tully J. G., Rose D. L., McCoy R. E., Carle P., Bové J. M., Whitcomb R. F. and Weisburg W. G.: *Mycoplasma melaleucae* sp. nov., a sterol-requiring mollicute from flowers of several tropical plants. *Int. J. Syst. Bact.* **40** (1990) 143–147.
48. Raederstorff D. and Rohmer M.: Sterol biosynthesis via cycloartenol and other biochemical features related to photosynthetic phyla in the amoebae *Naegleria*

lovaniensis and *Nagleria gruberi*. *Eur. J. Biochem.* **164** (1987) 427–434.
49. Raederstorff D. and Rohmer M.: The action of the systemic fungicides tridemorph and fenpropimorph on sterol biosynthesis by the soil amoeba *Acanthamoeba polyphaga*. *Eur. J. Biochem.* **164** (1987) 421–426.
50. Kochert G.: Sexual pheromones in algae and fungi. *A. Rev. Plant Physiol.* **29** (1978) 461–486.
51. Bolander F.: *Molecular Endocrinology*. Academic Press, New York (1989) pp. 261–289.
52. Sandor T. and Mehdi A. Z.: Steroids and evolution. In *Hormones and Evolution* (Edited by E. W. J. Barrington). Academic Press, New York, Vol. 1 (1979) pp. 1–72.
53. Clever U.: Gene activity patterns and cellular differentiation. *Am. Zoo.* **6** (1966) 33–41.
54. Richards G. and Ashburner M.: Insect hormones and the regulation of genetic activity. In *Biological Regulation and Development* (Edited by R. F. Goldberger and K. R. Yamamoto). Plenum, New York, Vol 3B (1984) pp. 213–253.
55. Schopf J. W.: The evolution of the earliest cells. *Sci. Am.* **239** (1978) 110–138.
56. Csaba G.: Development of hormone receptors: why do hormone receptors arise? *Experientia* **42** (1986) 715–718.
57. Csaba G.: Presence and development of hormone receptors in unicellular organisms. In *Progress In Comparative Endocrinology* (Edited by A. Epple, C. G. Scanes and M. H. Stetson). Wiley-Liss, New York (1990) pp. 116–126.
58. Hanke W.: Corticosteroid function: evolutionary aspects. In *Progress in Comparative Endocrinology* (Edited by A. Epple, C. G. Scanes and M. H. Stetson). Wiley-Liss, New York (1990) 445–452.
59. Tata J. R.: The search for the mechanism of hormone action. *Perspect. Biol. Med.* **29** (1986) S184–S204.
60. Tata J. R.: What is so unique about hormone action? *Molec. Cell. Endocr.* **36** (1984) 17–27.
61. Buckmann D.: The phylogeny and the polytropy of hormones. *Horm. Metab. Res.* **15** (1983) 211–217.
62. Tomkins G. M.: The metabolic code. *Science* **189** (1975) 760–763.
63. Chrousos G. P., Renquist D., Brandon D., Eil C., Pugeat M., Vigersky R., Cutler G. B. Jr., Loriaux D. L. and Lipsett M. B.: Glucocorticoid hormone resistance during primate evolution: receptor-mediated mechanisms. *Proc. Natn. Acad. Sci. U.S.A.* **79** (1982) 2036–2040.
64. Chrousos G. P., Loriaux D. L., Brandon D., Tomita M., Vingerhoeds A. C. M., Merriam G. R., Johnson E. O. and Lipsett M. B.: Primary cortisol resistance: a familial syndrome and an animal model. *J. Steroid Biochem.* **19** (1983) 567–575.
65. Pugeat M. M., Chrousos G. P., Nisula B. C., Loriaux D. L., Brandon D. and Lipsett M. B.: Plasma cortisol transport and primate evolution. *Endocrinology* **115** (1984) 357–361.
66. Schumacher M.: Rapid membrane effects of steroid hormones: an emerging concept in neuroendocrinology. *Trends Neurosci.* **13** (1990) 359–362.
67. Thomas P. and Meizel S.: Phosphatidylinositol 4,5-bisphosphate hydrolysis in human sperm stimulated with follicular fluid or progesterone is dependent upon Ca^{2+} influx. *Biochem. J.* **264** (1989) 539–546.
68. Touchette N.: Man bites dogma: a new role for steroid hormones. J. NIH Res. **2** (1990) 71–74.
69. Harrison N. L., Majewska M. D., Harrington J. W. and Barker J. L.: Structure-activity relationships for steroid interaction with the γ-aminobutyric acid^A receptor complex. *J. Pharmac. Exp. Ther.* **241** (1987) 346–353.
70. Majewska M. D.: Steroid and brain activity: essential dialogue between body and mind. *Biochem. Pharmac.* **36** (1987) 3781–3788.
71. Gee K. W., Bolger M. B., Brinton R. E., Coirini H. and McEwan B. S.: Steroid modulation of the chloride ionophore in rat brain: structure-activity requirements, regional dependence and mechanism of action. *J. Pharmac. Exp. Ther.* **246** (1988) 803–812.
72. Vincens M., Shu C., Maguilewsky M. and Philibert D.: A progesterone metabolite enhances the activity of the $GABA_A$ receptor complex at the pituitary level. *Eur. J. Pharmac.* **168** (1989) 15–21.
73. Brann D. W., Putnam C. D. and Mahesh V. B.: γ-Aminobutyric $acid_A$ receptors mediate 3α-hydroxy-5α-pregnan-20-one-induced gonadotropin secretion. *Endocrinology* **126** (1990) 1854–1859.
74. Belelli D., Lan N. C. and Gee K. W.: Anticonvulsant steroids and the GABA/benzodiazepine receptor-chloride ionophore complex. *Neurosci. Biobehav. Rev.* **14** (1990) 315–322.
75. Ke F. C. and Ramirez V. D.: Membrane mechanism mediates progesterone stimulatory effect on LHRH release from superfused rat hypothalmi *in vitro*. *Neuroendocrinology* **45** (1987) 514–517.
76. Pauling L. and Delbruck M.: The nature of the intermolecular forces operative in biological processes. *Science* **92** (1940) 79.
77. Watson J. and Crick F. H.: Molecular structure of nucleic acids. *Nature* **171** (1953) 737.