Progress in Molecular and Subcellular Biology

VOLUME 2

Complexes of Biologically Active Substances with Nucleic Acids and Their Modes of Action

Progress in Molecular and Subcellular Biology

2

Proceedings of the Research Symposium on

Complexes of Biologically Active Substances with Nucleic Acids and Their Modes of Action

Held at the Walter Reed Army Institute of Research
Washington, 16—19 March 1970

With 158 Figures

Springer-Verlag Berlin · Heidelberg · New York 1971

ISBN 3-540-05321-2 Springer-Verlag Berlin Heidelberg New York
ISBN 0-387-05321-2 Springer-Verlag New York Heidelberg Berlin

 Library of Congress Catalog Card Number 75-79748.
Printed in Germany. Typesetting and printing: Carl Ritter & Co., Wiesbaden.
Binding: Karl Hanke, Düsseldorf

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Complexes of Biologically Active Substances with Nucleic Acids — Yesterday, Today, Tomorrow

Fred E. Hahn

I. Introduction

The Symposium whose Proceedings are contained in this volume has been convened after a period of intensive research and increasing knowledge concerning the formation and structures of complexes of biologically active small molecules with nucleic acids and the biological, biochemical and pharmacological effects caused by such complex formation.

Firstly, the decade has seen many detailed investigations of the modes and mechanisms of action of clinical or experimental drugs which form complexes with nucleic acids, especially with DNA, and produce their antiprotozoal, antibacterial, antiviral and antineoplastic effects by interfering with processes in which nucleic acids participate. During this era the term "molecular pharmacology" has been adopted for the field of learning which concerns itself with drug action at the molecular level; knowledge of nucleic acid complexing drugs constitutes perhaps the most advanced area in molecular pharmacology. One result of this advancement is the progressive rationalization of previously empirical structure-activity relationships which provides information for drug design or molecular modification of existing prototype drugs with a view to improving chemotherapy. For example, our (Hahn, this volume) recognition that complex formation with DNA represents the basis of the antimalarial action of quinine has not only explained structure-activity rules which had been discovered some 30 years ago, but also explains the strong antiplasmodial effects of synthetic quinoline methanols which have been designed after quinine and has opened the door to a systematic exploration of this class of potentially useful drugs.

Secondly, the mutagenic effects of aminoacridines or of ethidium bromide led to the discovery of frame-shift mutations in chromosomal genes or of mitochondrial mutations, and have been explained through studies of the binding of these substances to DNA. But the same chemicals, foremost quinacrine, also act as antimutagens (De Courcy; Bach, this volume) which decrease the frequency with which bacteria or plasmodia mutate to resist the action of pharmacopeial chemotherapeutic drugs. We are probably at the beginning of an era in practical chemotherapy in which antimutagens will be administered along with chemotherapeutic drugs to prevent the emergence of microbial drug resistance in patients under therapy. Furthermore, the elimination of episomal resistance factors from enteric bacteria by acridines, known as the "curing" effect, opens the prospect of eliminating R-factor-mediated drug resistance after it has been established through R-factor transfer.

Thirdly, many carcinogenic substances form complexes with DNA. The study of this phenomenon is pursued in several laboratories (for example, LESKO, this volume) in the hope that mechanisms of chemical carcinogenesis will be elucidated and that the nature of genetic lesions, leading to neoplasia, will become understood. This understanding may be one prerequisite to the development of effective antineoplastic drugs.

Finally, it is apparent that substances which complex with DNA and prevent RNA transcription are simple models of genetic repressors. Not only is the flexibility of microorganisms in their response to changing nutritional environments based upon an interplay of repression and derepression on the regulatory segments of coordinated gene clusters, called operons, but the understanding of the developmental biology of higher organisms at the molecular level of organization and causation also depends upon the knowledge of the suspension or actuation of genetic potentials. It is at this point of conceptualization that progress in the study of DNA-complexing substances becomes progress in molecular biology.

The Walter Reed Army Institute of Research owes its original establishment in 1893 and many of its accomplishments to the concern with problems of communicable diseases. The Institute has sponsored this Symposium, whose Proceedings are published here, with the expectation that the knowledge and perspectives presented will ultimately be translated into tangible advances in chemotherapy. The time is approaching when scientifically premeditated drug design will supplant empirical search procedures.

II. Prehistory: Before the Double Helix

Research on the binding of low-molecular ligands to nucleic acids began in the 1940ies and was concerned with cytological staining characteristics of basic dyes. In a classical study, MICHAELIS (1947) recorded the effects produced by nucleic acids in the absorption spectra of basic dyes which "stain" DNA or RNA. His distinction between α-, β- and γ-bands of absorption established physical criteria for the binding of individual molecules, dimers or aggregates of dyes to nucleic acids. Since many DNA-complexing drugs have visible or ultraviolet absorption spectra, the same criteria also are useful in the study of nucleic acid complexes with drugs. MICHAELIS (1947) also anticipated the intercalation hypothesis of dye or drug binding to DNA by speculating that in dye-DNA complexes "each dye cation, combined with one phosphate group, must lie in the space between the planes of the pyrimidine or purine rings".

Interest in cytological staining specificity also led to studies by KURNICK and MIRSKY (1950) of the stoichiometry of the reaction of methyl green with DNA. The stable DNA-methyl green complex was introduced as an experimental substrate for the determination of the activities of deoxyribonucleases (KURNICK, 1950). We have begun to measure the rates of displacement of methyl green from DNA by DNA-complexing drugs and to consider different rates as functions of different affinities of the displacing compounds for DNA.

Unrelated, at the time, to the topic of nucleic acid complexes were extensive investigations by ALBERT and his associates (reviewed by ALBERT, 1968) on the relationships between the structures of aminoacridines and other N-heterocyclic

amines and their antibacterial activities. Aminoacridines had been introduced in 1913 by BROWNING as antibacterials for wounds. ALBERT, RUBBO and BURVILL (1949) recognized as essential structural requirements of N-heterocyclic amines to exert antibacterial activity, that such compounds must possess planar areas of 28 $Å^2$ or larger and substituted amino groups which are ionized to at least 50% at physiological pHs. These empirically derived structure-activity rules are now retrospectively recognized as the structural requirements for intercalation binding of antimicrobial substances to double-helical DNA.

III. History: After the Double Helix

WATSON and CRICK proposed the double-helical structure for DNA (1953) based on X-ray diffraction studies in the laboratory of WILKINS, model-building experiments and the logical requirement that DNA's structure must provide a determinant for its correct replication, i.e. for genetic continuity. While it is evident that knowledge of the macromolecular architecture of DNA provides the basis of determining structures of DNA complexes with low-molecular ligands, studies on such complexes were not promptly undertaken after the DNA model had been proposed. Work on the interaction of aminoacridines with DNA by PEACOCKE and SKERRETT (1956) was stimulated by the antibacterial properties of these compounds and measured the extent of binding of proflavine to DNA by spectrophotometric titration and equilibrium dialysis. The study emphasized the need for purines in DNA to bind proflavine and, in reiterating the structure-activity rules of ALBERT et al. (1949), considered them for the first time to be requirements for interaction with DNA; it also distinguished between one strong binding process by which one proflavine molecule is bound per approximately 5 nucleotides and a weaker process which involves the attachment of aggregates of the aminoacridine to DNA.

In comparing the influence of cationic polymers on the tendency of acridine orange to "stack" along such linear macromolecules, BRADLEY and WOLF (1959) concluded that the "stacking coefficient" of DNA was small by comparison to that of single-stranded polynucleotides, of heparin or of polyphosphate. STONE and BRADLEY (1961), in elaborating on these results, concluded that the aggregation of acridine orange, owing to dye-dye interactions, was a function of the conformation of the polymer to which the dye was bound and that the "stacking coefficient" for double-stranded DNA was smaller than for denatured DNA. This work, nevertheless, proposed the first model of the structure of a DNA-ligand complex which was based upon a consideration of the macromolecular architecture of the double helix.

Stimulated by the mutagenic action of aminoacridines and the carcinogenic action of certain benzacridines, LERMAN (as reviewed in 1964) undertook a series of studies on the structure of DNA-acridine complexes which have explained the strong (type 1) binding of aminoacridines to DNA (PEACOCKE and SKERRETT, 1956) and the frame-shift mutagenesis by aminoacridines (CRICK, BARNETT, BRENNER and WATTS-TOBIN, 1961) by postulating the intercalation model. Applying a set of hydrodynamic, optical and organic-chemical criteria, it was shown that substituted acridines become inserted between the levels of base pairs into double-helical DNA; the spaces for these insertions are created by local untwisting of the double helix by an estimated 12° of

rotation which causes a separation between previously adjacent base pairs of approximately 3.5 Å without a disturbance in the pattern of hydrogen bonds. The resulting lengthening of linear DNA has been measured radioautographically in electron micrographs (Cairns, 1962) and has suggested that only every second space between base pairs is available for intercalation. This is in accord with the stoichiometry of strong binding processes for proflavine (Peacocke and Skerrett, 1956) or chloroquine (Stollar and Levine, 1963) of one intercalant molecule per 4 to 5 component bases of DNA.

The original intercalation model of Lerman or its modification by Pritchard, Blake and Peacocke (1966) is also based upon the knowledge of the macromolecular architecture of DNA and, indeed, could not have been completely developed before the postulation of the DNA model by Watson and Crick (1953).

IV. Antibiotics

In 1960 Kirk, as well as Rauen, Kersten and Kersten, reported studies on the mode of action actinomycin D, the prototype of a series of antibiotics discovered by Waksman and Woodruff (1940). Actinomycin was found to form a complex with DNA; it cosediments with DNA and its absorption spectrum is altered by DNA. The antibiotic acts as a template poison and inhibits, preferentially, the transscription of RNA from DNA (Kirk, 1960; Hurwitz, Furth, Malamy and Alexander, 1962). Extensive studies on the DNA-actinomycin complex (reviewed by Reich, Cerami and Ward, 1967), and especially X-ray studies of the structure of the complex (Hamilton, Fuller and Reich, 1963) led to the proposal of a structural model in which actinomycin is lodged in the minor groove of the double helix and requires the amino group in position 2 of guanine or 2-aminopurine for binding to DNA. Müller and Crothers (1968) have extensively reinvestigated the properties of DNA complexes with a series of actinomycins and concluded that the hetero-tricyclic chromophore of the antibiotic is intercalated into DNA. The idea of intercalation of actinomycin has been fortified (Waring, this volume) by demonstrating typical conformational changes in supercoiled DNA upon reacting with the antibiotic; the absolute guanine requirement for binding of actinomycin to DNA is placed into doubt by studies of Wells (this volume).

Many other antibiotics form complexes with DNA, such as daunomycin, cinerubin, nogalamycin, chromomycin, mithramycin and olivomycin (as reviewed in Gottlieb and Shaw, 1967), echinomycin (Ward, Reich and Goldberg, 1965), quinoxaline antibiotics (Sato, Shiratori and Katagiri, 1967), hedamycin and rubiflavin (White and White, 1969), kanchanomycin (Friedman, Joel and Goldberg, 1969), anthramycin (Horwitz, this volume), sibiromycin (Gause, this volume) and distamycin (Krey and Hahn, 1970).

An interesting feature of the binding of antibiotics to DNA is the role of Mg^{++} in the binding process. For some substances, Mg^{++} causes the dissociation of their complexes with DNA, while for others it is an essential requirement for complex formation.

A few antibiotics react with DNA through the formation of covalent bonds. Mitomycin C and some of its congeners (reviewed by Szybalski and Iyer, 1967) are reduced *in vivo* and also can be reduced experimentally *in vitro* to active metabolites

with very short half-lifes; these metabolites condense with DNA and form covalent cross-links in the double helix. This cross-linked DNA is incapable of serving as a template for its own replication since the component strands can not undergo separation. For this reason, the mitomycins are specific inhibitors of DNA biosynthesis. The structural changes produced in DNA by cross-linking with reduced mitomycin are manifested by spontaneous renaturation of DNA whose linked component strands realign "in register". The detailed structure of the mitomycin-DNA complex is not yet known.

Anthramycin (KOHN and SPEARS, 1970) also forms a covalent bond with DNA. The nature of the chemical reaction and of the bonds which are formed are also unknown, but the principal biochemical effect of this complex formation is an inhibition of the DNA-dependent RNA and DNA polymerase reactions *in vitro* and of nucleic acid biosynthesis in anthramycin-exposed bacteria (HORWITZ, this volume).

V. Synthetic Drugs

While most antibiotics which form complexes with DNA are primarily of investigative interest, certain synthetic drugs of clinical importance exert their chemotherapeutic action also by binding to DNA. This has been studied for quinacrine (KURNICK and RADCLIFFE, 1962), a compound which LERMAN selected for some of his key experiments (1963) to test the intercalation hypothesis of the DNA-acridine complex. Quinacrine acts as a DNA template poison and inhibits the DNA-dependent DNA and RNA polymerase reactions (HAHN et al., 1966); the drug is either bacteriostatic or bactericidal depending upon its concentration, and inhibits, preferentially, DNA biosynthesis *in vivo* (CIAK and HAHN, 1967). A structurally related antimicrobial nitroacridine acts in a similar manner (WOLFE, this volume).

Chloroquine also binds to DNA (HAHN et al., 1966; YIELDING, this volume) by intercalation (O'BRIEN, ALLISON and HAHN, 1966; WARING, this volume), inhibits the DNA-dependent DNA and RNA polymerase reactions *in vitro* and, prominently, DNA biosynthesis in plasmodia (POLET and BARR, 1968). While antimalarial 8-aminoquinolines also bind to DNA (HOLBROOK, this volume), they evidently do not form intercalation complexes, and their modes of action have remained unknown. Since it has been suggested that these drugs are converted *in vivo* into chemotherapeutically active metabolites, the meaning of *in vitro* observations of their binding to DNA is not clear.

The antischistosomal drug, miracil D, which also has antibacterial and antitumor activity binds to DNA, probably by intercalation, and inhibits specifically the transcription of RNA from DNA, i.e. RNA biosynthesis (WEINSTEIN, this volume).

Synthetic drugs of lesser medical importance such as ethidium bromide (WARING; WAGNER; MAHLER, this volume) and quinoline methanols (HAHN, this volume) also form complexes with DNA.

VI. Alkaloids

The first studies of binding of alkaloids to DNA were carried out, beginning in 1964, by MAHLER and his associates (cited by HAHN, this volume) on a group of steroidal diamines. This work was undertaken because these compounds possess, at

neutral pH, two positive charges with a fixed separation corresponding to the interval between two DNA phosphates across the minor groove of the double helix. Like alipathic diamines of similar spacing of charges, steroidal diamines stabilize DNA to heat. Additionally, these compounds have offered the unique opportunity of studying conformational changes which they produce in DNA by optical methods since their absorption spectra do not occlude the absorption maximum of DNA at 259 nm. One of these substances, irehdiamine, has a warped and non-planar ring structure which appears to eliminate intercalation binding to DNA from consideration; yet, this alkaloid has been found (Waring, this volume) to produce conformational transitions in superhelical DNA which are typical for intercalation.

A second set of studies on DNA-alkaloid complexes was suggested by the antimalarial action of quinine and by the presumed antimalarial properties of berberine and colchicine. Quinine and berberine were found to form complexes with DNA (Hahn, this volume). This explains the effects of quinine on plasmodial DNA biosynthesis and, hence, on the development of schizonts (Polet and Barr, 1968) as well as the curative action of berberine in cutaneous leishmaniasis and its effect as a mitochondrial mutagen. On the other hand, standard experimental tests for complex formation with DNA were consistently negative for colchicine, and the mutual effects of colchicine and DNA upon each other's specific rotation (Ilan and Quastel, 1966) remain unexplained.

Quite recently the binding of the hallucinogenic ergot alkaloid, lysergic acid diethylamide, to DNA (Wagner; Yielding, this volume) has been investigated in the hope that this will explain the induction of chromosomal damage in lymphocytes by LSD.

Since alkaloids, in general, are organic amines, it can be expected that additional members of this class of natural compounds will be found to bind to DNA, if only by ionic attraction.

VII. Superhelical DNA

The past four years have seen the emergence of knowledge concerning the wide distribution in nature of a form of circular DNA which is twisted into supercoils because it has a built-in deficiency in the number of helical turns. At the time of the Symposium, 45 different superhelical DNAs were known. These occur in animal viruses, in bacterial viruses, in mitochondria, in bacterial episomes, and in the cytoplasm of animal cells. Neither the mechanism of biosynthesis of superhelical DNAs nor their biological role are understood at this time.

Intercalation binding of synthetic drug or antibiotics (Waring; Bauer, this volume) produces characteristic conformational transitions in superhelical DNA. Progressive intercalation causes progressive unwinding of superhelices by gradual compensation for the natural deficiency in helical turns; at a defined equivalence point, superhelical DNA will have been converted into ordinary circular DNA. Further intercalation, producing further increments in helical turns, twists this circular DNA into unnatural supercoils which owe their existence not to a *deficiency* but to an *excess* of helical turns. If one were to speculate that the supercoiled condition represents a DNA storage form with suspended template function, he might assume that, for example, the antitprypanosomal action of ethidium bromide which leads to the

formation of akinetoplastic trypanosomes, results from a suspension of the function of kinetoplastic DNA. It is not impossible that the selective toxicity of intercalative drugs in eliminating bacterial episomes, is not the result of an indiscriminate and massive occupancy of all DNA, but, in contrast, the selective effect of such drugs of changing the conformation of circular episomal DNA and of tying it up into artificial and non-functional supercoils (HAHN and CIAK, 1971).

VIII. The Future

Two structural models of DNA complexes with low-molecular substances have been considered. One involves lateral attachment of such compounds to DNA and is exemplified by the "stacking model" of BRADLEY and WOLF (1959) or by the binding of spermine and other polyamines to the double helix (HERBST, this volume). The other is the intercalation model of LERMAN (1964). With the exception of a few X-ray diffraction studies (HAMILTON et al., 1963; NEVILLE and DAVIES, 1966; LIQUORI, COSTANTINO, CRESCNEZI, ELIA, GIGLIO, PULITI, DE SANTIS SAVINO and VITAGLIANO, 1967; SUWALSKY, TRAUB, SHMUELI and SUBIRANA, 1969), knowledge of the structures of DNA complexes has been produced by statistical rather than deterministic experiments and interpretations.

Investigating a series of low-molecular complexing agents with variations in a given prototype structure (LERMAN, 1964; MÜLLER and CROTHERS, 1968) exemplifies the extent of determinism which has been attained.

Two new approaches, however, promise to identify the specific binding sites for drugs on DNA as well as the structures of the DNA-drug complexes formed. One of these approaches (WELLS, this volume) uses duplex deoxyribopolynucleotides with monotonously repeated base sequences, i.e. it systematically varies the covalent structure of the binding polymer instead of the structure of a prototype ligand which is bound. This approach is capable of considerable extension and refinement, including the use of duplex oligomers with linear sequences of seven or eight nucleotides, i.e. of the critical length for one complete helical turn in DNA.

The other approach is the study of binding of drugs to DNA by nuclear magnetic resonance spectroscopy as exemplified by the work of DANYLUK and VICTOR (1970) on the interaction of actinomycin and DNA. The NMR spectrum of DNA shows an unresolved continuum of signals, but the signals from a binding drug molecule undergo specific changes from that of the free drug depending upon which reactive groups of the drug are involved in interaction with DNA. Preliminary work on the NMR spectrum of chloroquine (VICTOR, this volume) provides the basis for such DNA binding studies.

The unambiguous determination of structures of DNA-drug complexes might not only explain the chemotherapeutic and genetic effects of biologically active substances which form complexes with DNA, but should logically furnish essential information for the premeditated design of substances whose biological target is DNA, and whose biological actions should be predictable. It may well be that the first breakthrough in chemotherapy research to the premeditated design of effective drugs will occur in this area of molecular pharmacology.

References

Albert, A.: Selective toxicity, 4. ed. London: Methuen 1968.

— Rubbo, S. D., Burvill, M. I.: The influence of chemical constitution on antibacterial activity. Part IV: A survey of heterocyclic bases with special reference to benzquinolines, phenanthridines, benzacridines, quinolines and pyridines Brit. J. exp. Path. **30**, 159 (1949).

Bradley, D. F., Wolf, M. K.: Aggregation of dyes bound to polyanions Proc. nat. Acad. Sci. (Wash.) **45**, 944 (1959).

Cairns, J.: The application of radioautography to the study of DNA viruses Cold Spr. Harb. Symp. quant. Biol. **27**, 311 (1962).

Ciak, J., Hahn, F. E.: Quinacrine (Atebrin): Mode of action. Science **156**, 655 (1967).

Crick, F. H. C., Barnett, L., Brenner, S., Watts-Tobin, R. J.: General nature of the genetic code for proteins Nature (Lond.) **192**, 1227 (1961).

Danyluk, S. S., Victor, T. A.: The structure and molecular interactions of actinomycin D, quantum aspects of heterocyclic compounds in chemistry and biochemistry, II. The Israeli Academy of Sciences Humanities, p 394. Jerusalem 1970.

Friedman, P. A., Joel, P. B , Goldberg, I. H.: Interaction of kanchanomycin with nucleic acids. I. Physical properties of the complex. Biochemistry **8**, 1535 (1969).

Gottlieb, D., Shaw, P. D., Eds.: Antibiotics I. Mechanism of action. Berlin-Heidelberg-New York: Springer 1967.

Hahn, F. E., Ciak, J.: Elimination of bacterial episomes by DNA-complexing compounds. Ann. N.Y. Acad. Sci. (1971) (in press).

— O'Brien, R. L., Ciak, J., Allison, J. L., Olenick, J. G.: Studies on modes of action of chloroquine, quinacrine and quinine and on chloroquine resistance. Milit. Med. **131**, 1071 (1966).

Hamliton, L., Fuller, W., Reich, E.: X-ray diffraction and molecular model building studies of the interaction of actinomycin with nucleic acids. Nature (Lond.) **198**, 538 (1963).

Hurwitz, J., Furth, J. J., Malamy, M., Alexander, M.: The role of deoxyribonucleic acid in ribonucleic acid synthesis, III. The inhibition of the enzymatic synthesis of ribonucleic acid and deoxyribonucleic acid by actinomycin D and proflavin. Proc. nat. Acad. Sci. (Wash.) **48**, 1222 (1962).

Ilan, J., Quastel, J. H.: Effects of colchicine on nucleic acid metabolism during metamorphosis of *Tenebrio molitor* and in some mammalian tissues. Biochem. J. **100**, 448 (1966).

Kirk, J. M.: The mode of action of actinomycin D. Biochim. biophys. Acta (Amst.) **42**, 167 (1960).

Kohn, K. W., Spears, C. L.: Reaction of anthramycin with deoxyribonucleic acid. J. molec. Biol. **51**, 551 (1970).

Krey, A. K., Hahn, F. E.: Studies on the complex of distamycin A with calf thymus DNA. FEBS Letters **10**, 175 (1970).

Kurnick, N. B.: Determination of desoxyribonuclease activity by methyl green; application to serum. Arch. Biochem. **29**, 41 (1950).

— Mirsky, A. E.: Methyl green-pyronine. II. Stoichiometry of reaction with nucleic acids. J. gen. Physiol. **33**, 265 (1950).

— Radcliffe, I. E.: Reaction between DNA and quinacrine and other antimalarials. J. Lab. clin. Med. **60**, 669 (1962).

Lerman, L. S.: The structure of the DNA-acridine complex. Proc. nat. Acad. Sci. (Wash.) **49**, 94 (1963).

— Acridine mutagens and DNA structure. J. cell. comp. Physiol. **64**, Suppl. 1, 1 (1964).

Liquori, A. M., Costantino, L., Crescenzi, V., Elia, V., Giglio, E., Puliti, R., de Santis Savino, M., Vitagliano, V.: Complexes between DNA and polyamines: A molecular model. J. molec. Biol. **24**, 113 (1967).

Michaelis, L.: The nature of the interaction of nucleic acids and nuclei with basic dyestuffs. Cold Spr. Harb. Symp. quant. Biol. **12**, 131 (1947).

Müller, W., Crothers, D. M.: Studies on the binding of actinomycin and related compounds to DNA. J. molec. Biol. **35**, 251 (1968).

Neville, D. M., Davies, D. R.: The interaction of acridine dyes with DNA: An x-ray diffraction and optical investigation. J. molec. Biol. **17**, 57 (1966).

O'Brien, R. L., Allison, J. L., Hahn, F. E.: Evidence for intercalation of chloroquine into DNA. Biochim. biophys. Acta (Amst.) **129**, 622 (1966).

Peacocke, A. R., Skerrett, J. N. H.: The interaction of aminoacridines with nucleic acids. Trans. Faraday Soc. **52**, 261 (1956).

Polet, H., Barr, C. F.: Chloroquine and dihydroquinine. In vitro studies of their antimalarial effect upon *Plasmodium knowlesi*. J. Pharmacol. exp. Ther. **164**, 380 (1968).

Pritchard, N. J., Blake, A., Peacocke, A. R.: Modified intercalation model for the interaction of aminoacridines and DNA. Nature (Lond.) **212**, 1360 (1966).

Rauen, H. M., Kersten, H., Kersten, W.: Zur Wirkungsweise von Actinomycinen. Z. physiol. Chem. **321**, 139 (1960).

Reich, E., Cerami, A., Ward, D. C.: Actinomycin. In: Antibiotics I. Mechanism of action. Berlin-Heidelberg-New York: Springer 1967.

Sato, K., Shiratori, O., Katagiri, K.: The mode of action of quinoxaline antibiotics. Interaction of quinomycin A with deoxyribonucleic acid. J. Antibiot. (Tokyo) Ser. A. **20**, 270 (1967).

Stollar, D., Levine, L.: Antibodies to denatured deoxyribonucleic acid in lupus erythematosus serum. V. Mechanism of DNA-anti DNA Inhibition by chloroquine. Arch. Biochem. **101**, 335 (1963).

Stone, A. L., Bradley, D. F.: Aggregation of acridine orange bound to polyanions: The stacking tendency of deoxyribonucleic acids. J. Amer. chem. Soc. **83**, 3627 (1961).

Suwalski, M., Traub, W., Shmueli, U., Subirana, J. A.: An X-ray study of the interaction of DNA with spermine. J. molec. Biol. **42**, 363 (1969).

Szybalski, W., Iyer, V. N.: The mitomycins and porfiromycins. In: Antibiotics I. Mechanism of action. Berlin-Heidelberg-New York: Springer 1967.

Waksman, S. A., Woodruff, H. B.: Bacteriostatic and bactericidal substances produced by a soil actinomyces. Proc. Soc. expt. Biol. (N.Y.) **45**, 609 (1940).

Ward, D. C., Reich, E., Goldberg, I. H.: Base specificity in the interaction of polynucleotides with antibiotic drugs. Science **149**, 1259 (1965).

Watson, J. D., Crick, F. H. C.: The structure of DNA. Cold Spr. Harb. Symp. quant. Biol. **18**, 123 (1953).

White, H. L., White, J. R.: Hedamycin and rubiflavin complexes with deoxyribonucleic acid and other polynucleotides. Biochemistry **8**, 1030 (1969).