PROGRESS IN

Nucleic Acid Research and Molecular Biology

edited by

WALDO E. COHN

KIVIE MOLDAVE

Volume 31

PROGRESS IN
Nucleic Acid Research and Molecular Biology

edited by

WALDO E. COHN

Biology Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

KIVIE MOLDAVE

Department of Biological Chemistry
College of Medicine
University of California
Irvine, California

Volume 31

1984

ACADEMIC PRESS, INC.

(Harcourt Brace Jovanovich, Publishers)

Orlando San Diego New York London
Toronto Montreal Sydney Tokyo

ACADEMIC PRESS, INC.
Orlando, Florida 32887

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1 7DX

LIBRARY OF CONGRESS CATALOG CARD NUMBER: 63-15847

ISBN 0-12-540031-4

PRINTED IN THE UNITED STATES OF AMERICA

84 85 86 87 9 8 7 6 5 4 3 2 1

Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

JANET ANDERSEN (161), *Department of Microbiology, School of Medicine, SUNY at Stony Brook, Stony Brook, New York 11794*

JOHN M. BOYLE (1), *Paterson Laboratories, Manchester M20 9BX, England*

C. THOMAS CASKEY (295), *Howard Hughes Medical Institute Laboratories and Departments of Biochemistry and Medicine, Baylor College of Medicine, Houston, Texas 77030*

A. CRAIG CHINAULT (295), *Howard Hughes Medical Institute Laboratories and Departments of Biochemistry and Medicine, Baylor College of Medicine, Houston, Texas 77030*

FRANCIS S. COLLINS (315), *Departments of Human Genetics, Medicine, and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510*

NICHOLAS DELIHAS (161), *Department of Microbiology, School of Medicine, SUNY at Stony Brook, Stony Brook, New York 11794*

MÅNS EHRENBERG (191), *Department of Molecular Biology, Biomedical Center, S-751 24 Uppsala, Sweden*

HELGA KERSTEN (59), *Institute of Physiological Chemistry, The University of Erlangen-Nürnberg, Nürnberg, Federal Republic of Germany*

C. G. KURLAND (191), *Department of Molecular Biology, Biomedical Center, S-751 24 Uppsala, Sweden*

RADHA K. MANDAL (115), *Department of Biochemistry, Bose Institute, Calcutta 700 009, India*

SATYAPRIYA SARKAR (267), *Department of Muscle Research, Boston Biomedical Research Institute, and Department of Neurology, Harvard Medical School, Boston, Massachusetts 02114*

RAM P. SINGHAL (161), *Chemistry Department, Wichita State University, Wichita, Kansas 67208*

PAUL T. STRICKLAND (1), *NCI Frederick Cancer Research Facility, Frederick, Maryland 21701*

ALBERT J. WAHBA (221), *Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi 39216*

SHERMAN M. WEISSMAN (315), *Departments of Human Genetics, Medicine, and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut 06510*

CHARLES L. WOODLEY (221), *Department of Biochemistry, University of Mississippi Medical Center, Jackson, Mississippi 39216*

Abbreviations and Symbols

All contributors to this Series are asked to use the terminology (abbreviations and symbols) recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (CBN) and approved by IUPAC and IUB, and the Editor endeavors to assure conformity. These Recommendations have been published in many journals (*1*, *2*) and compendia (*3*) in four languages and are available in reprint form from the Office of Biochemical Nomenclature (OBN), as stated in each publication, and are therefore considered to be generally known. Those used in nucleic acid work, originally set out in section 5 of the first Recommendations (*1*) and subsequently revised and expanded (*2*, *3*), are given in condensed form (I–V) below for the convenience of the reader. Authors may use them without definition, when necessary.

I. Bases, Nucleosides, Mononucleotides

1. *Bases* (in tables, figures, equations, or chromatograms) are symbolized by Ade, Gua, Hyp, Xan, Cyt, Thy, Oro, Ura; Pur = any purine, Pyr = any pyrimidine, Base = any base. The prefixes S–, H_2, F–, Br, Me, etc., may be used for modifications of these.

2. *Ribonucleosides* (in tables, figures, equations, or chromatograms) are symbolized, in the same order, by Ado, Guo, Ino, Xao, Cyd, Thd, Ord, Urd (Ψrd), Puo, Pyd, Nuc. Modifications may be expressed as indicated in (1) above. Sugar residues may be specified by the prefixes r (optional), d (=deoxyribo), a, x, l, etc., to these, or by two three-letter symbols, as in Ara-Cyt (for aCyd) or dRib-Ade (for dAdo).

3. *Mono-, di-, and triphosphates of nucleosides* (5′) are designated by NMP, NDP, NTP. The N (for "nucleoside") may be replaced by any one of the nucleoside symbols given in II-1 below. 2′-, 3′-, and 5′- are used as prefixes when necessary. The prefix d signifies "deoxy." [Alternatively, nucleotides may be expressed by attaching P to the symbols in (2) above. Thus: P-Ado = AMP; Ado-P = 3′-AMP] cNMP = cyclic 3′:5′-NMP; Bt_2cAMP = dibutyryl cAMP, etc.

II. Oligonucleotides and Polynucleotides

1. Ribonucleoside Residues

(a) Common: A, G, I, X, C, T, O, U, Ψ, R, Y, N (in the order of I-2 above).

(b) Base-modified: sI or M for thioinosine = 6-mercaptopurine ribonucleoside; sU or S for thiouridine; brU or B for 5-bromouridine; hU or D for 5,6-dihydrouridine; for isopentenyl; f for formyl. Other modifications are similarly indicated by appropriate *lower-case* prefixes (in contrast to I-1 above) (*2*, *3*).

(c) Sugar-modified: prefixes are d, a, x, or l as in I-2 above; alternatively, by *italics* or boldface type (with definition) unless the entire chain is specified by an appropriate prefix. The 2′-*O*-methyl group is indicated by *suffix* m (e.g., -Am- for 2′-*O*-methyladenosine, but -mA- for 6-methyladenosine).

(d) Locants and multipliers, when necessary, are indicated by superscripts and subscripts, respectively, e.g., -m_2^6A- = 6-dimethyladenosine; -s^4U- or -^{4}S- = 4-thiouridine; -ac^{4}Cm- = 2′-*O*-methyl-4-acetylcytidine.

(e) When space is limited, as in two-dimensional arrays or in aligning homologous sequences, the prefixes may be placed *over the capital letter*, the suffixes *over the phosphodiester symbol*.

2. Phosphoric Residues [left side = 5′, right side = 3′ (or 2′)]

(a) Terminal: p; e.g., pppN . . . is a polynucleotide with a 5′-triphosphate at one end; Ap is adenosine 3′-phosphate; C > p is cytidine 2′:3′-cyclic phosphate (*1*, *2*, *3*); p < A is adenosine 3′:5′-cyclic phosphate.

(b) Internal: hyphen (for known sequence), comma (for unknown sequence); unknown sequences are enclosed in parentheses. E.g., pA-G-A-C(C_2,A,U)A-U-G-C > p is a sequence with a (5′) phosphate at one end, a 2′:3′-cyclic phosphate at the other, and a tetranucleotide of unknown sequence in the middle. (Only codon triplets should be written without some punctuation separating the residues.)

3. Polarity, or Direction of Chain

The symbol for the phosphodiester group (whether hyphen or comma or parentheses, as in 2b) represents a 3′-5′ link (i.e., a 5′ . . . 3′ chain) unless otherwise indicated by appropriate numbers. "Reverse polarity" (a chain proceeding from a 3′ terminus at left to a 5′ terminus at right) may be shown by numerals or by right-to-left arrows. Polarity in any direction, as in a two-dimensional array, may be shown by appropriate rotation of the (capital) letters so that 5′ is at left, 3′ at right when the letter is viewed right-side-up.

4. Synthetic Polymers

The complete name or the appropriate group of symbols (see II-1 above) of the repeating unit, enclosed in parentheses if complex or a symbol, is either (a) preceded by "poly," or (b) followed by a subscript "n" or appropriate number. No space follows "poly" (2, 5).

The conventions of II-2b are used to specify known or unknown (random) sequence, e.g.,

polyadenylate = poly(A) or A_n, a simple homopolymer;

poly(3 adenylate, 2 cytidylate) = poly(A_3C_2) or $(A_3,C_2)_n$, an *irregular* copolymer of A and C in 3 : 2 proportions;

poly(deoxyadenylate-deoxythymidylate) = poly[d(A-T)] or poly(dA-dT) or $(dA\text{-}dT)_n$ or $d(A\text{-}T)_n$, an *alternating* copolymer of dA and dT;

poly(adenylate,guanylate,cytidylate,uridylate) = poly(A,G,C,U) or $(A,G,C,U)_n$, a random assortment of A, G, C, and U residues, proportions unspecified.

The prefix copoly or oligo may replace poly, if desired. The subscript "n" may be replaced by numerals indicating actual size, e.g., $A_n \cdot dT_{12\text{-}18}$.

III. Association of Polynucleotide Chains

1. *Associated* (e.g., H-bonded) chains, or bases within chains, are indicated by a *center dot* (not a hyphen or a plus sign) separating the *complete* names or symbols, e.g.:

poly(A) · poly(U) or $A_n \cdot U_m$
poly(A) · 2 poly(U) or $A_n \cdot 2U_m$
poly(dA-dC) · poly(dG-dT) or $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_m$.

2. *Nonassociated* chains are separated by the plus sign, e.g.:

$2[\text{poly(A)} \cdot \text{poly(U)}] \rightarrow \text{poly(A)} \cdot 2\,\text{poly(U)} + \text{poly(A)}$
or $2[A_n \cdot U_m] \rightarrow A_n \cdot 2U_m + A_n$.

3. Unspecified or unknown association is expressed by a comma (again meaning "unknown") between the completely specified chains.

Note: In all cases, each chain is completely specified in one or the other of the two systems described in II-4 above.

IV. Natural Nucleic Acids

RNA	ribonucleic acid or ribonucleate
DNA	deoxyribonucleic acid or deoxyribonucleate
mRNA; rRNA; nRNA	messenger RNA; ribosomal RNA; nuclear RNA
hnRNA	heterogeneous nuclear RNA
D-RNA; cRNA	"DNA-like" RNA; complementary RNA

mtDNA	mitochondrial DNA
tRNA	transfer (or acceptor or amino-acid-accepting) RNA; replaces sRNA, which is not to be used for any purpose
aminoacyl-tRNA	"charged" tRNA (i.e., tRNA's carrying aminoacyl residues); may be abbreviated to AA-tRNA
alanine tRNA or $tRNA^{Ala}$, etc.	tRNA normally capable of accepting alanine, to form alanyl-tRNA, etc.
alanyl-tRNA or alanyl-$tRNA^{Ala}$	The same, with alanyl residue covalently attached. [*Note*: fMet = formylmethionyl; hence $tRNA^{fMet}$, identical with $tRNA_f^{Met}$]

Isoacceptors are indicated by appropriate subscripts, i.e., $tRNA_1^{Ala}$, $tRNA_2^{Ala}$, etc.

V. Miscellaneous Abbreviations

P_i, PP_i	inorganic orthophosphate, pyrophosphate
RNase, DNase	ribonuclease, deoxyribonuclease
t_m (not T_m)	melting temperature (°C)

Others listed in Table II of Reference 1 may also be used without definition. No others, with or without definition, are used unless, in the opinion of the editor, they increase the ease of reading.

Enzymes

In naming enzymes, the 1978 recommendations of the IUB Commission on Biochemical Nomenclature (*4*) are followed as far as possible. At first mention, each enzyme is described *either* by its systematic name *or* by the equation for the reaction catalyzed *or* by the recommended trivial name, followed by its EC number in parentheses. Thereafter, a trivial name may be used. Enzyme names are not to be abbreviated except when the substrate has an approved abbreviation (e.g., ATPase, but not LDH, is acceptable).

References*

1. *JBC* **241**, 527 (1966); *Bchem* **5**, 1445 (1966); *BJ* **101**, 1 (1966); *ABB* **115**, 1 (1966), **129**, 1 (1969); and elsewhere.†
2. *EJB* **15**, 203 (1970); *JBC* **245**, 5171 (1970); *JMB* **55**, 299 (1971); and elsewhere.†
3. "Handbook of Biochemistry" (G. Fasman, ed.), 3rd ed. Chemical Rubber Co., Cleveland, Ohio, 1970, 1975, Nucleic Acids, Vols. I and II, pp. 3–59.
4. "Enzyme Nomenclature" [Recommendations (1978) of the Nomenclature Committee of the IUB]. Academic Press, New York, 1979.
5. "Nomenclature of Synthetic Polypeptides," *JBC* **247**, 323 (1972); *Biopolymers* **11**, 321 (1972); and elsewhere.†

Abbreviations of Journal Titles

Journals	*Abbreviations used*
Annu. Rev. Biochem.	ARB
Annu. Rev. Genet.	ARGen
Arch. Biochem. Biophys.	ABB
Biochem. Biophys. Res. Commun.	BBRC

*Contractions for names of journals follow.

†Reprints of all CBN Recommendations are available from the Office of Biochemical Nomenclature (W. E. Cohn, Director), Biology Division, Oak Ridge National Laboratory, Box Y, Oak Ridge, Tennessee 37830, USA.

Biochemistry	Bchem
Biochem. J.	BJ
Biochim. Biophys. Acta	BBA
Cold Spring Harbor	CSH
Cold Spring Harbor Lab.	CSHLab
Cold Spring Harbor Symp. Quant. Biol.	CSHSQB
Eur. J. Biochem.	EJB
Fed. Proc.	FP
Hoppe-Seyler's Z. physiol. Chem.	ZpChem
J. Amer. Chem. Soc.	JACS
J. Bacteriol.	J. Bact.
J. Biol. Chem.	JBC
J. Chem. Soc.	JCS
J. Mol. Biol.	JMB
J. Nat. Cancer Inst.	JNCI
Mol. Cell. Biol.	MCBiol
Mol. Cell. Biochem.	MCBchem
Mol. Gen. Genet.	MGG
Nature, New Biology	Nature NB
Nucleic Acid Research	NARes
Proc. Nat. Acad. Sci. U.S.	PNAS
Proc. Soc. Exp. Biol. Med.	PSEBM
Progr. Nucl. Acid. Res. Mol. Biol.	This Series

Some Articles Planned for Future Volumes

Role of Editing in DNA Fidelity
MAURICE J. BESSMAN

Control of Transcription in SV40
GOKUL C. DAS, S. K. NIYOGI, AND N. SALZMAN

ATP-Ubiquitin-Dependent Degradation of Intracellular Proteins
AVRAM HERSHKO

Chemical Changes Induced in DNA by Gamma Rays
FRANKLIN HUTCHINSON

The Role of tRNA Anticodon Triplet and Loop in the Specific Interaction with Aminoacyl-tRNA Synthetases
LEV L. KISSELEV

Interactive Three-Dimensional Computer Graphics in Protein and Genetic Engineering
ROBERT LANGRIDGE

The Photobiology and Radiobiology of *Micrococcus radiodurans*
R. E. MOSELEY

Ribosomal Translocation: Facts and Models
ALEXANDER S. SPIRIN

Sequence-Specific Affinity Modification of Nucleic Acids or Reactive Derivatives of Oligo- and Polynucleotides in Molecular Biological Investigations
VALENTIN V. VLASSOV AND D. G. KNORRE

Contents

Structure, Function, and Evolution of 5-S Ribosomal RNAs

Nicholas Delihas, Janet Andersen, and Ram P. Singhal

Optimization of Translational Accuracy

C. G. Kurland and Måns Ehrenberg

Molecular Aspects of Development in the Brine Shrimp *Artemia*

Albert J. Wahba and Charles L. Woodley

Translational Control Involving a Novel Cytoplasmic RNA and Ribonucleoprotein

Satyapriya Sarkar

The Hypoxanthine Phosphoribosyltransferase Gene: A Model for the Study of Mutation in Mammalian Cells

A. Craig Chinault and C. Thomas Caskey

The Molecular Genetics of Human Hemoglobin

Francis S. Collins and Sherman M. Weissman

Immunoassay of Carcinogen-Modified DNA

PAUL T. STRICKLAND

NCI-Frederick Cancer Research Facility
Frederick, Maryland

JOHN M. BOYLE

Paterson Laboratories
Manchester, England

The development of immunological assays for the detection of damaged and undamaged nucleic acids has advanced rapidly in recent years. This approach, which complements existing biochemical and physical approaches, provides a valuable tool for investigators in the field of nucleic acid research. Early progress in the production of antisera specific for photodamaged and undamaged DNA and for normal and rare nucleotides has been summarized in a symposium (*1*) and in general reviews (*2–4*). More recent reviews discuss immunological detection of radiation (UV[1] and ionizing) damage in DNA (*5*), of chemical damage in DNA (*6, 7*), and of naturally occurring modified nucleosides (*8*).

[1] Abbreviations: AAF, acetamidofluorene; AFB_1, aflatoxin B_1; BPDE, benzo[*a*]pyrene-7,8-diol 9,10-epoxide; BSA, bovine serum albumin; ELISA, enzyme-linked immunoadsorbent assay; FA, Freund's adjuvant; FI, fluorescein isothiocyanate; Ig, immunoglobulin; KLH, keyhole limpet hemocyanin; RIA, radioimmunoassay; RIST, radioimmunosorbent technique; SLE, systemic lupus erythematosus; USERIA, ultrasensitive enzymatic RIA; UV, ultraviolet (radiation).

Progress in Nucleic Acid Research and Molecular Biology, Vol. 31

ISBN 0-12-540031-4

A major advantage of quantitative immunoassays for DNA damage is that the DNA or chemical adduct under investigation does not have to be labeled. This is particularly useful for analysis of DNA from tissue in which the cells are slowly proliferating and therefore cannot be labeled at high specific activity and also in cases where a radioactive tag is required on a compound (e.g., a carcinogen) to detect its binding to DNA. In the former case, the sensitivity (level of detection) of some assays depends on the specific activity of the DNA; in the latter case, the particular compound may be difficult and/or expensive to label. In addition, several methods (immunofluorescence, immunoperoxidase, immunoautoradiography) allow the observation of DNA damage and repair in cells and tissue sections *in situ* (*9–14*). This is particularly useful when heterogeneous cell populations are being studied, and chemical extraction and subsequent analysis of DNA allow only the average amount of damage sustained by the whole cell population to be measured. Immunological assay of damage in individual cells and/or cell types can be used to determine how DNA damage is distributed within a cell population (or tissue), and may provide information unobtainable by other means.

In this review we concentrate on the application of immunological methods for determining the quantity and distribution of various modified DNA structures, particularly those that are potentially mutagenic or carcinogenic. Many of these methods are similar to those used for detecting specific structures in undamaged DNA, and they have been well described in an earlier review (*8*), which may be read as a companion to the present paper. In Section I, we describe methods of special relevance to antibodies against DNA adducts and mention newer techniques, such as hybridoma technology, introduced since the earlier review. Similarly, Section II is intended, in part, to update this work and provide a context for discussing antibodies to DNA adducts.

I. Methodology

A. Immunogens

In general, the methods used to produce antibodies specific for carcinogen-altered nucleosides or polynucleotides are adapted from studies with unaltered or naturally occurring modified nucleic acid components (*8, 15, 16*). Like other haptens, nucleosides and polynucleotides must be linked to carrier proteins to render them immuno-

genic in animals, and this is accomplished either by covalent coupling in the case of nucleosides or by electrostatic coupling in the case of polynucleotides.

The most widely used procedure for covalent coupling is that in which the ribose group of the nucleoside is oxidized at positions C-2 and C-3 by periodate; the resulting carbonyl groups react spontaneously with free amino groups of the protein (*17*, *18*). The bond is then stabilized with sodium borohydride. This coupling procedure has been used successfully to raise antibodies against a number of carcinogen-modified ribonucleosides (Sections III–V). Although the procedure can be used only with ribonucleosides, this has not been a serious problem since antibodies raised against ribonucleoside–protein conjugates react equally well (or better) with the analogous deoxyribonucleosides.

Several methods exist for coupling deoxyribonucleosides to carrier proteins. They can be oxidized in the presence of a platinum catalyst (*19*) to 5′-carboxylic acids, which can then be coupled to the carrier protein by means of a carbodiimide (*20*). Alternatively, deoxyribonucleoside 5′-monophosphates or -diphosphates can be linked to proteins by means of water-soluble carbodiimides (*21*, *22*).

Polynucleotides, on the other hand, can easily be complexed with methylated bovine serum albumin (BSA). The original rationale for using methylated BSA·polynucleotide complexes for immunization was quite simple (*1*). Albumin had often been used as a protein carrier for simple haptens (and for nucleosides), and it was possible to produce a basic form of this protein by methylation of the negatively charged exposed carboxyl groups. The mBSA binds electrostatically to the acidic DNA polymer and forms stable complexes. Nucleic-acid·mBSA immunizing complexes have been used to raise antibodies to a variety of damaged polynucleotides, including UV-irradiated DNA (*23*), photooxidized DNA (*24*), acetamidofluorene-damaged DNA (*25*), benzo[*a*]pyrene-damaged DNA (*26*), and aflatoxin B-damaged DNA (*27*).

Although relatively pure samples of carcinogen-modified nucleosides can be prepared for linkage to proteins, polynucleotides treated with carcinogens always contain a mixture of modified and unmodified nucleotides. The level of modification of the polynucleotide can influence the specificity of the immunogenic response (*28*). Modification in the range of 1 to 10% has been used successfully with various carcinogens (*7*, *27*, *29*).

The choice of an appropriate immunogen (nucleoside or polynucleotide), depends on how the DNA is to be treated (*30*). If the modi-

fied DNA moiety of interest is to be detected in hydrolyzed DNA samples (degraded to nucleosides), the preferred immunogen would be the specific damaged nucleoside conjugated with a carrier protein. However, if the damaged moiety is to be detected in intact DNA, the preferred immunogen may be a protein conjugate of DNA treated with an agent capable of inducing the required modification. The latter method will, in most cases, produce a heterogeneous range of damage characteristic of the damaging agent that will give rise to antisera containing antibodies of corresponding specificities. As a result, the antibodies may have to be purified for specific forms of damage (by affinity chromatography) or produced by hybridoma methods to ensure monoclonal origin (and monospecificity).

Both methylated and unmethylated BSA are widely used carrier proteins for nucleic acid haptens and produce quite satisfactory results. A few studies have been undertaken to find more effective carrier proteins for carcinogen-damaged DNA. One of these studies compared BSA, rabbit serum albumin, and keyhole limpet hemocyanin (KLH) for their ability to render O^6-methyldeoxyguanosine (O^6-MedGuo) immunogenic in rabbits (*31*). KLH proved to be the best carrier of the three on the basis of highest antibody affinity constant and lowest degree of cross-reactivity with unaltered dGuo. This finding was attributed to the high molecular weight (800,000) of KLH and its dissimilarity to the proteins of the immunized animal. Similarly, methylated KLH was found to be superior to mBSA as a carrier for aflatoxin B_1-damaged DNA (*28*). However, KLH is not universally superior to BSA (*31a*), and the choice of carrier may be affected also by the nature of the hapten and, possibly, the strain of animal immunized.

B. Immunization Schedules

Injection schedules for immunizing rabbits with nucleic acid haptens vary considerably among laboratories; however, a general approach that has been described as "fast-release" (*6*) is fairly common (*15*). Nucleoside·protein conjugate (0.5–5.0 mg per rabbit) emulsified in complete Freund's adjuvant (FA) is injected at subcutaneous, intradermal, or intramuscular sites or in hind footpads. Additional weekly or biweekly injections of conjugate emulsified in incomplete FA are given at the same sites or intravenously, and animals are bled 5–10 days after final injection.

A second approach, described as slow-release (*6, 31*), uses nucleoside–protein conjugate immobilized by adsorption onto aluminum hydroxide. This complex is emulsified with complete FA and injected

(0.5 mg per rabbit) into hind footpads and many (~50) intradermal sites. Eight weeks later the animals are given booster injections by the same procedure. After another 8 weeks, a second booster is administered by intramuscular injection of conjugate (0.5 mg per rabbit) emulsified in incomplete FA. This schedule was found to be superior to the fast-release schedule on the basis of higher titer and higher affinity constant of the antibodies induced (*31*).

Immunization of mice for the production of monoclonal antibodies requires the same immunogens as those used in rabbits; however, different doses of immunogen and, in some cases, different injection sites are used. When quantities of immunogen are limited, a slow-release approach can be useful. Intradermal injections of conjugate (20 μg) adsorbed onto aluminum hydroxide and emulsified in complete FA are administered and repeated 3 and 6 months later, followed 2 weeks later by intraperitoneal injection (*30*). When immunogen is plentiful, higher doses of conjugate (50–200 μg) emulsified in complete FA can be so administered at 2–3-week intervals (*27, 29*). At this stage, serum samples are assayed for antibody titer to eliminate animals with low responses, and a final intravenous booster of conjugate without FA is given 3–4 days before fusion of spleen cells.

A typical immunization schedule for raising antibodies against thymine photoproducts (mainly thymine dimers) is shown in Table I. Generally BALB/c mice are used for immunization because the myeloma cell lines commonly used in hybridoma production are derived from this strain, but (C57B1/6 × BALB/c)F_1 mice have also been used and may produce higher serum titers than BALB/c mice (*28*).

C. Monoclonal Antibodies

The methods used to produce monoclonal antibodies specific for carcinogen-modified haptens are identical to those used for other hap-

TABLE I
IMMUNIZATION PROCEDURE

Week	Treatment[a]	Injection site[b]
1	UVssDNA/mBSA (100 μg) + CFA	i.p.
3	UVssDNA/mBSA (200 μg) + ICFA	i.p.
5	UVpoly(dT)/mBSA (75 μg) + ICFA	i.p.
7	UVpoly(dT)/mBSA (50 μg)	i.v.
7 + 4 days	Fuse spleen cells	

[a] mBSA, methylated bovine serum albumin; CFA, Freund's adjuvant; ICFA, incomplete CFA.

[b] i.p., intraperitoneal; i.v., intravenous (tail vein).