MASTER

DISTRIBUTION OF THIS DOCUMENT UNLIMITED

12/7

#C00-3521-126

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

PROGRESS REPORT
TO
THE UNITED STATES ATOMIC ENERGY COMMISSION
CONTRACT AT(30-1)910
BIOLOGICAL EFFECTS OF RADIATION AND RELATED BIOCHEMICAL
AND PHYSICAL STUDIES

Proposal #5: Chemical and Biological Studies on
Nucleic Acids and Derivatives
Contract Period: January 1, 1971 to December 31, 1971
Report Period: July 1, 1970 to June 30, 1971
Investigators: George B. Brown, Ph.D., Member
Aaron Bendich, Ph.D., Member
Department: Divisions of Biological Chemistry
and Cell Biochemistry

The Sloan-Kettering Institute for Cancer Research
410 East 68th Street, New York, New York 10021

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

September 30, 1971

MASTER

DISTRIBUTION OF THIS DOCUMENT UNLIMITED

George B. Brown, Ph.D., Head, Section 5701

ONCOGENIC PURINE N-OXIDES

Our attention has been focused on recent evidence that the 3-acyloxypurine 8-substitution reaction is accompanied, in aqueous solution at room temperature and neutral pH, by a free-radical reaction mechanism, and on the possible relationship of that to our earlier reports of the photochemical production of free-radicals from 3-hydroxyxanthine and related derivatives. With two modes of formation of reactive intermediates, an ionic mechanism and a radical mechanism, decision must be reserved as to which may be responsible for initiation of the cancer process.

The recent Development Section of the 21-Yr Report (p. 9) outlines the metabolic and chemical studies which furnish the background for the present emphasis on photochemically induced rearrangements and free-radical formation.

RECENT PHOTOCHEMICAL STUDIES (~1969-71)

Photochemistry of N-Hydroxypurines.— Earlier studies on the photochemistry of purine N-oxides have indicated that alterations in the pH of aqueous media can influence the course of the photochemical reactions. This change may result from an alteration in the tautomeric species accompanying ionization of protons associated with the N-oxide or N-hydroxy function. With compounds whose order of ionization and pKa's are known, and solutions at selected pH's which afford the maximum amount of each pure ionic species, we are delineating the photochemical reactivities of each ionic species. With this reference information we then plan to proceed to investigate which specific excited state can lead to each product from the various ionic species.

1

2

1-Hydroxyhypoxanthine (1) was shown to exist predominantly in the 6-oxo-1-N-hydroxy form in the neutral species. Its first ionization, $pK_a = 5.68$, causes a notable change in the uv spectrum

and takes place from the 1-hydroxyl group to produce an enolate anion containing a conjugated N-oxide function. This modification of the chromophore results in a change of the photochemical products (Table 1). At pH 3.0 in the presence of O_2 and with

Table I: Photolysis of 1-Hydroxyhypoxanthine

Run	Solvent & pH	Filter	Time	%Xanthine	%Hx	%Recovery of S.M.*
1	H ₂ O pH 3.0	Corex	15 min	1.7	20	24 ^a
2	" "	"	120 "	0	6.0	0 ^a
3	" "	"	120 "	8.5	40	0
4	" pH 8.5	"	60 "	18	5.5	0
5	" "	"	60 "	16	6.8	0
6	(H ₂ O-NH ₃) pH 13	"	60 "	19	5.5	2.4
7	CH ₃ OH	"	90 "	11	17	5.8

a/ Without degassing

* Recovery of starting material based on O.D.

a corex filter (260 nm cut off) 1 was rapidly (15 min) deoxygenated to yield mainly hypoxanthine (20%). Some rearrangement to xanthine (1.7%) was also noted. Longer irradiation time caused severe loss of material (Run 2), consequently degassing with N_2 was employed routinely thereafter. With degassing (Run 3) the yield of both deoxygenation (40%) and rearrangement (8.5%) products increased. At pH 8.5, where the main resonance form is the enolate N-oxide, reaction was faster and the rearrangement to xanthine ($17 \pm 1\%$) was favored. Further ionization of 1 in the imidazole ring ($pK_a = 10.20$) did not cause a significant change in the photochemical pathways (Run 6). Irradiation of 1 in CH₃OH (Run 7) where the neutral species should predominate gave results comparable to those in water at pH 3 (Run 3).

Table II: Photolysis of 7-Methyl-1-Hydroxyhypoxanthine

Run	Solvent	pH	Filter	Time	%7-CH ₃ Xan	%7-CH ₃ Hx	% S.M
8	H ₂ O	3.0	Corex	80 min	1.1	28	0
9	H ₂ O	8.5	"	15 "	9.1	17	0
10	CH ₃ OH	-	"	47 "	7.1	42	0
11	C ₂ H ₅ OH	-	"	15 "	4.6	57	0
12a	CH ₃ OH*	-	"	20 "	21	12.4	0
12b	"	-	"	20 "	10	5.7	0
12c	"	-	"	20 "	15	10.2	0
12d	"	-	"	20 "	12.4	12.4	0
Average for (12a-d)					14	10	0
13	CH ₃ CN	-	"	30 "	2.3	74	0
14	p-dioxane	-	"	45 "	0	70	0

* CH₃OH was dried over Molecular Sieve 3A

The 7-methyl derivative of 1 was also investigated (Table II) and gave results in aqueous solutions parallel to those of 1. Irradiation of the neutral species of 1-hydroxy-7-methylhypoxanthine (2) at pH 3 (Run 8) produced mainly deoxygenation (28%) with some rearrangement (1%). At pH 8.5 (Run 9) the monoanion of 2 ($pK_a = 5.63$) gave a higher yield of xanthine (9.1), but a significant amount of deoxygenation (17%) was still observed. Irradiation of 2 in several organic solvents (Runs 10-14) gave a predominance of deoxygenation (40-70%), usually with some accompanying rearrangement (0.7%). When CH₃OH was dried over molecular sieve type 3A, the amounts of deoxygenation and rearrangement were reversed, with rearrangement predominating. This, the lower overall recovery, and other results from studies on the rearrangement of 3-acetoxanthine in sieve-dried CH₃OH suggest that the basicity of the molecular sieves was sufficient to induce ionization of 2. Thus we are studying the photochemical behavior of the monoanion, rather than the neutral species. In accord with this interpretation these results in CH₃OH (Runs 12a-d) parallel those of the monoanion in H₂O, pH 8.5 (Run 9).

Table III: Sensitization of 7-CH₃-1-OH-Hypoxanthine

Run	Solvent	Sensitizer(0.2M)	Filter	Time	%7-CH ₃ Xan	%7-CH ₃ Hx	% S.M.
15	CH ₃ CN		pyrex	30 min	0	5.1	92
16	"	acetophenone*	"	" "	0	65	0
17	"	benzophenone ⁺	"	40 "	0	87	3.8
18	"	fluorene [‡]	"	30 "	-	8	92
19	p-dioxane		"	30 "	0	5.8	92
20	"	---	"	240 "	0	26	35
21	"	acetophenone*	"	30 "	0	48	30
22	"	benzophenone ⁺	"	30 "	0	9.1	88
23	"	fluorene [‡]	"	30 "	0	3.1	80
24	acetone	(neat) acetone	"	30 "	0	78	0

* Triplet E = 76.6^a

+ " " = 68.5^a

‡ " " = 67.6^a

a/ Photochemistry, Calvert and Pitts.

The greater solubility of 2 in organic solvents has permitted photochemical studies with triplet sensitizers (Table III). In these studies a pyrex filter (cut off 280 nm) was used to eliminate light of the wavelength of the major chromophore of 2 at 257 nm. There was still some photochemical reaction of 2 in organic solvents, even with a pyrex filter (because 2 absorbs somewhat beyond 280), as shown by Runs 15, 19 and 20. The only reaction under these conditions was deoxygenation, but the yield was quite low (5-6% in 30 min) and there was little loss of material. The addition of triplet sensitizers, however, greatly increased the yield and rate of deoxygenation. In CH₃CN the addition of acetophenone (³E=73.6 Kcal/mole) gave 65% deoxygenation in 30 min (Run 16), while benzophenone (³E=68.5 Kcal/mole) gave an 87% yield in 40 min (Run 17). The addition of fluorene (³E=67.6 Kcal) to the CH₃CN solution gave results similar to the irradiation without sensitizers. Those results demonstrate that the deoxygenation of 2 proceeds through the triplet state. The slower reaction with benzophenone (68.5 Kcal) and the lack of sensitization by fluorene in CH₃CN indicate that the energy level of the triplet state in 2 is ca. 68 Kcal/mole. Similar results were obtained in p-dioxane, in which added acetophenone, benzophenone and fluorene gave yields of 7-methyl-hypoxanthine of 48, 9 and 3%, respectively, in 30 min (Runs 21-23). This compares to 5.8% with no sensitizer (Run 19). These data support those in CH₃CN and emphasize the requisite energy level of the triplet sensitizer for the deoxygenation reaction. In

acetone alone ($E=84$ Kcal/mole), the deoxygenation of 2 proceeds in good yield (Run 24).

This result is paralleled by the photochemical reaction of 1 in the presence of acetone (Runs 25-27) (Table IV). Since 1 is poorly soluble in acetone alone, best results were obtained in acetone-H₂O (1:1) (Run 27). A 63% yield of hypoxanthine was obtained in this case in 60 min.

Table IV: Sensitization of 1-Hydroxyhypoxanthine

Run	Solvent	Sensitizer	Filter	Time	%Xan	%Hx	%1-OH-Hx
25	Acetone ^a	(neat)	pyrex	60 min	0	6.3	84
26	"	"	"	30 "	0	2.0	95
27	H ₂ O ^b	Acetone(50%)	"	60 "	0	63%	14%

a/ Some 1-hydroxyhypoxanthine was not soluble.

b/ A small unknown peak was also observed.

Using a pyrex filter resulted in higher overall recoveries of material than using a corex filter. An exception to this were the runs using acetophenone as a triplet sensitizer. It may be that the triplet energy level of acetophenone is sufficient to catalyze further reactions of either 2 or of 7-methylhypoxanthine with oxygen atoms produced from the deoxygenation of 2. This would represent a form of photodynamic degradation and needs to be examined more thoroughly.

Table V: Quenching Studies of 7-CH₃-1-OH-Hx

Run	Solvent	Quencher	Filter	Time	%CH ₃ Xan	%CH ₃ Hx	%S.M.
28	CH ₃ CN	---	corex	30 min	2.3	74	0
29	"	piperylene (1.0 M)	"	" "	1.4	47	15
30	CH ₃ OH	---	"	20 "	14	10	0
31	"	O ₂ (saturated)	"	" "	14	5.2	0

Triplet quenching studies (Table V) with piperylene in CH₃CN (Run 29) or O₂ in CH₃OH (Run 31) gave less convincing evidence than the sensitizer experiments, but the results are

in qualitative agreement with the conclusion that deoxygenation proceeds through the triplet state.

In conclusion, the major photochemical reaction of the neutral species of both 1-hydroxyhypoxanthine (1) and 1-hydroxy-7-methylhypoxanthine (2), which exist predominantly as the N-hydroxy tautomer in the neutral species is deoxygenation to the parent purine. Some rearrangement of the oxygen to the adjacent carbon was also observed from the neutral species in water, alcohols and even in CH_3CN , but this was not observed in p-dioxane. Ionization of the N-hydroxy function causes the appearance of a new chromophore at 220 nm, reflecting the formation of an enolate anion containing an N-oxide function. This change in resonance form is reflected in the photochemical reactivity by a small increase in the rearrangement reaction, a large decrease in the deoxygenation reaction and a significantly poorer overall recovery of material.

Studies on these N-hydroxyhypoxanthine derivatives, with both triplet sensitizers and quenchers, indicate that deoxygenation proceeds through the triplet state, of the N-hydroxy tautomer which has an energy level >68 Kcal/mole.

Photochemistry of N-Oxide Tautomers of N-Oxidized Purines.- In addition to studies of compounds which exist predominantly in the N-hydroxy tautomer, parallel studies have been undertaken on several compounds which exist mainly as the N-oxide tautomer. Irradiation of 6-methylpurine 1-oxide ($\text{pK}_a=7.5$) yields both rearrangement and deoxygenation products. At pH 3 some 4-amino-5-acetylimidazole is also noted; it probably results from the hydrolysis of an intermediate on the rearrangement pathway. The results to date are summarized in Table VI. In water the major reaction with low pressure Hg (253 nm) light is rearrangement to the 2-oxopurine (Runs 1 and 2). This reaction is accelerated in CH_3OH (Runs 3 and 4). In CH_3CN with a pyrex filter to exclude light below 280 nm, a reversal of the extent of the two photochemical processes is noted and rapid deoxygenation becomes the predominant reaction (Run 6).

Table VI: Irradiation of 6-Methylpurine 1-Oxide

Run	Solvent	pH	Filter	Lamp	Time	% 2-OH 6-Me purine	% 6-Me purine	% S.M.	% 4-NH ₂ 5-acetyl imidazole
1	H ₂ O	3.0	--	Low Hg.	24 hr	17	2.3	48	26%
2	H ₂ O	9.5	--	"	24 hr	32	4.6	19	--
3	CH ₃ OH	--	--	"	5 "	10	2	23	--
4	CH ₃ OH(N ₂)	--	--	"	15 min	26	0	14	--
5	CH ₃ OH(N ₂)	--	pyrex	High Hg.	15 "	6.3	8.5	55	--
6	CH ₃ CN(N ₂)	--	pyrex	"		8.8	91%	0	--

To exclude the possibility of N-oxide-N-hydroxy tautomerism, a compound constrained in the N-oxide tautomeric form, 6,9-dimethylpurine-1-oxide, was prepared.

In acid solution (pH 3) this gave several photochemical products, including the rearranged 2-hydroxy derivative, the deoxygenated purine, the ring opened imidazole and a fourth unidentified product (3). In alkaline solution, and in CH₃OH, EtOH and CH₃CN only rearrangement and deoxygenation products were obtained in addition to unidentified product (3). The photoproduct 3, when heated in acid solution, is converted to 2-hydroxy-6,9-dimethylpurine, which suggests that 3 is an intermediate in the rearrangement process. An attractive possibility for the structure of 3 is the oxazirane compound 3:

These preliminary results indicate that both deoxygenation and rearrangement can proceed from the N-oxide tautomer. The reactions are sensitive to a change in the medium and to the wavelength of the exciting light.

SOLID STATE IRRADIATION OF N-HYDROXY PURINES

We have found that uv irradiation (254 nm) of 3-hydroxyxanthine or 3-hydroxyguanine in the solid state will produce a color change from white to purple and will generate a free-radical, as determined by ESR. This radical is stable in the solid state, but is rapidly destroyed by H₂O, CH₃OH or DMSO. From such solutions the major product of the irradiation has been identified as the deoxygenated

purine, xanthine or guanine. In the case of 3-hydroxyxanthine a time study of the irradiation showed that the radical content increased nearly linearly for the first 100 minutes of irradiation, became stable at about 12% radical and showed but slight increase with continued irradiation. The yield of xanthine increased more slowly and did not level off until over 200 hours of irradiation, with a maximum of 65%. The study also showed the presence of a second unidentified minor product which increases in amount with an increased duration of uv radiation.

We have also irradiated several N-methyl derivatives of 3-hydroxyxanthine and found that radicals are produced in each case, although in lower yield and without the purple color which accompanies the formation of a radical from 3-hydroxyxanthine. The ESR signal is not modified by the presence of methyl substituents at N-1 or N-7, but is changed significantly when methyl groups are present at N-9 and C-8. The radical must be associated with the 3-N-OH; xanthine does not yield a radical. The influence of methyl substituents on the ESR signal suggests interaction with the radical. Work is currently in progress to prepare the 8-deutero derivatives of several N-methyl-3-hydroxyxanthines to determine the interactions of the radical with the C-8 proton. We hope to determine the type of radical generated and the degree of interaction with N-3 and other portions of the purine ring system. The present evidence is indicative of an amidogen radical which is delocalized from N-3 throughout the purine π -system.

Dissolving the purple photo product in water discharges the purple color to produce a yellow solution with no ESR spectrum. In di-n-butyl sulfoxide the purple coloration is retained, but the ESR signal is lost. This suggests the purple color is not related to the radical, but may be associated with the second product noted on Dowex chromatography.

REFERENCES

1. Sugiyama, K.; Teller, M. N.; Parham, J. C. and Brown, G. B., Purine N-Oxides. XXXII. A comparison of the oncogenicities of 3-hydroxyxanthine guanine 3-N-oxide and some related compounds, Cancer Res. 30:184-188 (1970).
2. Stohrer, G. and Brown, G. B., Purine N-Oxides. XXXIII. Oncogenic purine derivatives: Evidence for a possible proximate oncogen, Science 167:1622-1624 (1970).
3. Giner-Sorolla, A.; Gryte, C.; Cox, M. L. and Parham, J. C., Purine N-Oxides. XXXIV. Synthesis of purine 3-oxide, 6-methylpurine 3-oxide and related derivatives, J. Org. Chem. 36:1228-1232 (1971).

Manuscripts in press

4. Birdsall, N. J. M.; Lee, T. C.; Delia, T. J. and Parham, J. C., Purine N-Oxides. XXXV. Alkylated guanine 3-oxides and 3-hydroxyxanthines, J. Org. Chem.
5. Parham, J. C.; Winn, T. G. and Brown, G. B., Purine N-Oxides. XXXVI. The tautomeric structures of the 3-N-oxides of xanthine and guanine, J. Org. Chem.
6. Birdsall, N. J. M.; Wolcke, U.; Parham, J. C. and Brown, G. B., Purine N-Oxides. XXXIX. N-Acetyl derivatives of N-hydroxyxanthines, Tetrahedron.
7. Birdsall, N. J. M.; Wolcke, U.; Lee, T.-C. and Brown, G. B., Purine N-Oxides. XL. The 3-acyloxypurine 8-substitution reaction: Scope: Syntheses of 8-substituted xanthines and guanines, Tetrahedron.
8. Birdsall, N. J. M.; Wolcke, U.; Parham, J. C. and Brown, G. B., Purine N-Oxides. XLI. The 3-acyloxypurine 8-substitution reaction: On the mechanism of the reaction, Tetrahedron.

Manuscripts in preparation

9. Zvilichovsky, G. and Brown, G. B., Purine N-Oxides. The cyclization of 4-amino-5-nitrouacil with formaldehyde. Preparation and properties of 7-hydroxyxanthine.
10. Parham, J. C.; Pullman, I. and Brown, G. B., Purine N-Oxides. Photochemically induced free-radicals from oncogenic purine N-oxides.

Note Drafts of 9 and 10 can be furnished if desired.

Abstracts

11. Stöhrer, G.; Corbin, E. and Brown, G. B., Metabolic activation of the oncogen 3-hydroxyxanthine, Proc. Am. Assoc. Cancer Res. 11:76 (1970).
12. Brown, G. B.; Stöhrer, G.; Sugiura, K. and Teller, M.N., Oncogenic purine derivatives, in International Cancer Congress, 10th, Houston, Texas, 1970. Abstracts. Austin, Texas, Medical Arts Publishing Co., 1970, p. 8.

Aaron Bendich, Ph.D., Chief

We have continued our examination of those embryonal antigens which appear in tumors of adult mice (1). Since they were found in each of 88 different cancers which arose spontaneously or were induced either by oncogenic chemicals, viruses, or ionizing radiations (2), study of these antigens may provide further understanding of carcinogenesis.

These antigens were present in fetal extracts in increasing amounts from the 9th day of gestation to parturition, decreasing to undetectable levels by the 5th week after birth. Examinations of 11 different strains of mice failed to reveal embryonal antigens in any normal adult tissues with one exception, skin, extracts of which cross-reacted with the anti-embryo sera used in our diagnostic tests. These cross-reacting skin antigens were distinguished and separated chromatographically from the embryonal, neonatal and tumor antigens on the basis of molecular weight differences. The antigens do not appear in rapidly proliferating tissues such as bone marrow or intestinal epithelium. The coincident tumor-embryo antigens are water soluble and appear to be proteins as judged by amino acid analysis. Fractionation of neonatal preparations on DEAE-Sephadex has yielded three distinct components separable by charge differences, all antigenically active and with molecular weights of about 67,000 (3).

In vitro propagation of three tumor-producing murine cell lines was accompanied by the loss of the tumor-embryo antigens which they originally contained. The antigens reappeared in the tumors which arose when the cultured cells were reinjected into appropriate hosts.

Attempts were made to uncover possible biological or biochemical activity in the tumor-embryo antigens. Precipitating antibodies were not detected in mice which received injections of the antigens, nor were the animals protected against challenges of tumor cells. However, the immunization schedule employed in these mice led to loss of large patches of body hair (3). The investigations are continuing.

The universal appearance of embryonal gene products in murine tumors supports the hypothesis of retrogenetic expression as a significant aspect of the cancer's cell biochemical development. In addition to the dormant embryonal activities which are turned on, adult hormone activities appear in certain non-metastatic tumors in tissues which do not have the relevant endocrine function. Further, deletion of certain normal tissue antigens in tumor cells also occurs. Accordingly, a picture which emerges from the combined studies is that a disruption of the orderly cellular genetic control mechanisms is a characteristic of the malignant change.

We have continued study (4-7) of some of the changes which attend the treatment of cells and animals with carcinogenic agents, such as 3,4-benzpyrene (BP) and 7,12-dimethylbenz(a)anthracene (DMBA), DNA extracted from other cells, and DNA to which ^3H -BP has been attached co-valently (the latter has been provided in a collaborative study with Dr. P.O.P. Ts'o). In our Progress Report (September 1970), we reported that Chinese hamster (CH) cells permanently acquired the ability to synthesize murine antigens following their treatment with DNA extracted from mouse Ehrlich ascites tumor cells (malignant potential was also acquired). The frequency of alteration was about 1 in 10^6 cells. We have examined methods to enhance the frequency and the most efficacious was to carry out the treatment in the presence of DEAE-D (diethylaminoethyl-dextran, a basic polymer which was reported to inhibit nuclease action and enhance pinocytosis). Although some toxicity resulting from the DEAE-D was manifest, frequency was increased about 100-fold. DEAE-D was labeled with ^3H to study the mechanism of its effect on DNA-cell interactions. Autoradiography revealed no significant uptake or cellular localization of the ^3H -DEAE-D (in the presence or absence of DNA), suggesting its effect was primarily on the DNA rather than on the cell. A complex formed since fibers containing both DNA and DEAE-D were obtained when these are suitably admixed. However, when various mammalian cells were co-cultured with such fibers containing ^3H -DNA, many of the cells in close contact showed a massive uptake of DNA. When CH cells were exposed to DEAE-D and CH DNA containing ^3H -BP in covalent linkage (base: BP = ca. 900), autoradiography revealed an occasional uptake of clusters of label in the cytoplasm, nucleus and nucleolar regions. The labeling pattern was quite different when either ^3H -BP or ^3H -DNA was used.

The biological effects of the uptake are under study.

Experiments were initiated to study the possible uptake and tissue distribution of DNA (labeled with ^3H , or with co-valently attached ^3H -BP) in mice and rats following intravenous and intraperitoneal injection. The distribution of label throughout the internal organs 24 hours following administration by either route was quite similar. The liver, spleen and lung, in that order, showed the greatest retention of radioactivity.

References

1. STONEHILL, E.H. and BENDICH, A., Retrogenetic Expression: The reappearance of embryonal antigens in cancer cells, *Nature* 228: 370-372 (1970).
2. STONEHILL, E.H., HIGGINS, P. and BENDICH, A., Distinction of normal adult antigens from the coincident antigens of tumor and embryo tissues, Abstract, 62nd Annual Meeting of the Proc. Amer. Assoc. for Cancer Res., Chicago, Ill., April 8-10, 1971, Vol. 12, p. 42, March 1971.
3. STONEHILL, E.H., BORENFREUND, E., and BENDICH, A., Anachronistic genetic expression recognized by the production of fetal antigens in cancer cells, Abstract, "Fetal Antigens in Cancer" Workshop and Conference at the Oak Ridge National Laboratory, Tenn., May 23-25, 1971; full paper to appear in a monograph of Symposium entitled "Fetal Antigens in Cancer", in press.
4. MARQUARDT, H., BENDICH, A., PHILIPS, F.S. and HOFFMANN, D., Binding of (G- ^3H)-7,12-dimethylbenz(a) anthracene to DNA of normal and of rapidly dividing hepatic cells of rats, *Chem.-Biol. Interactions* 3:1-11 (1971).
5. MARQUARDT, H., BENDICH, A. and PHILLIPS, F., DNA binding and synthesis in regenerating liver of rats given 7,12-dimethylbenz(a)anthracene (DMBA) during prereplicative phase, Abstract, 62nd Annual Meeting of the Proc. Amer. Assoc. for Cancer Res., Chicago, Ill., April 8-10, 1971, Vol. 12, p. 15, March 1971.

6. BORENFREUND, E., HONDA, Y., STEINGLASS, M. and BENDICH, A.,
Studies on DNA-induced heritable alteration of mammalian
cells, J. Exper. Med. 132:1071-1089 (1970).
7. BENDICH, A., BORENFREUND, E., and HONDA, Y., DNA-induced
heritable alteration of mammalian cells, Abstract,
Symposium: Uptake of Informative Molecules by Living Cells
Centre d'Etude de l'Energie Nucléaire C.E.N./S.C.K.,
Mol, Belgium, August 31-September 2, 1970; full paper
in press.